International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Issue 1, 2025

Original Article

# ADDITIVE CYTOTOXIC EFFECT OF CISPLATIN AND ANDROGRAPHIS PANICULATA EXTRACT THROUGH THE MODULATION OF APOPTOTIC MARKERS, CYCLIN-D AND VEGF EXPRESSIONS IN SKOV3 OVARIAN CANCER CELL LINE

**FARA VITANTRI DIAH[1,2*], NUZLI FAHDIA MAZFUFAH[3], WAWAIMULI AROZAL[4a], MELVA LOUISA[4b], SEPTELIA INAWATI WANANDI[5], SOMASUNDARAM ARUMUGAM[6], REMYA SREEDHAR[7], PUSPITA EKA WUYUNG[7]**

**[1]Doctoral Program in Medical Sciences, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia. [2]Department of Obstetrics and Gynecology, Syarif Hidayatullah State Islamic University Jakarta, Jakarta, Indonesia. [3]Stem Cell and Tissue Engineering Research Center, Indonesia Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. [4a,b]Department of Pharmacology and Therapeutics, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia. [5]Department of Biochemistry and Molecular Biology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia. [6]Department of Pharmacology and Toxicolgy, National Institute of Pharmaceutical Education and Research (NIPER), Kolkata, India. [7]Department of Pharmacy, Sister Nivedita University, Kolkata, India. [8]Department of Pathological Anatomy, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia**

**[*]Corresponding author: Fara Vitantri Diah; [*]Email: faravitantri@gmail.com**

***Received: 14 Sep 2024, Revised and Accepted: 20 Nov 2024***

**ABSTRACT**

**Objective:** This study aims to investigate the possibility of additive cytotoxic effects of cisplatin and *Andrographis paniculate (Burm. f.) Nees*(AP) via apoptotic, cell cycle and angiogenesis pathways.

**Methods:** CC50 cisplatin, AP and Andrographolide (AG) were determined by the cell viability of SKOV3 after its exposure to these substances. SKOV3 cells were then divided into 6 experimental groups: one negativecontrol group, one with CC50 cisplatin alone, and three where CC50 was combined with CC50 AP, ½CC50 AP, and 1.5CC50 AP, respectively. The additive cytotoxic effect of cisplatin with AP or AG was evaluated through the modulation of several pathways via qRT-PCR of their markers: apoptotic pathways indicated by Bax, BCL2, Caspase 3 and Caspase 9 expression; cell cycle indicated by Cyclin-D expression; angiogenesis pathways by VEGF expression.

**Results:** Cisplatin reduces cell viability to 54%, 37% when combined with AG, and 30%, 23% and 20% with ½CC50 AP, CC50 AP and 1.5CC50 AP, respectively. AG and AP extract decreases SKOV3 cell viability in a dose-dependent manner. Cisplatin combined with AP showed a statistically significant increase in BAX, Caspase 3, Caspase 9 expression and a decrease in BCL2, which indicated synergy in apoptotic pathways. The best result was seen in cisplatin combined with ½CC50 AP. A decrease in Cyclin D and VEGF was seen in all groups, the best seen in ½CC50 AP and CC50 AP, respectively, showing optimal cell cycle arrest and anti-angiogenesis properties when cisplatin is combined with AP extract.

**Conclusion:** Combining cisplatin with AP extract enhanced cell cycle arrest, apoptosis, and anti-angiogenesis properties.

**Keywords**: Andrographolide, Apoptosis, Angiogenesis, Cycle cell, Ovarian cancer, SKOV3


DOI: https://dx.doi.org/10.22159/ijap.2025v17i1.52654 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

## INTRODUCTION

Ovarian cancer ranks second as the cause of death among women due to gynecological cancers worldwide [1]. The incidence and mortality of ovarian cancer are still relatively high despite the development of current ovarian cancer treatments and screenings. WHO data from 2022 recorded a global incidence of 324,603 ovarian cancer cases, with a mortality rate reaching 206,956 [2]. The nonspecific symptoms of ovarian cancer make it rarely detected in early stages, often found at advanced stages with various organ metastases. Currently, the management of ovarian cancer consists of a combination of cytoreductive surgery and chemotherapy based on platinum (cisplatin) and taxanes. However, numerous issues such as chemotherapy resistance relapse cases, severe chemotherapy side effects, and low 5-year survival rates seem to indicate that existing ovarian cancer management is suboptimal in addressing the burden of this disease. One approach currently offered to answer these existing problems is combining existing standard ovarian cancer therapy with adjuvant therapy by using natural phytopharmaceuticals.

*Andrographis paniculata (Burm. f.) Nees (Acanthaceae)* (*A. paniculata*, Chuanxinlian) is a phytopharmaceutical containing main constituents such as diterpenoids, flavonoids, and polyphenols. This phytopharmaceutical has been used for a long time. It is well-known for its various pharmacological effects, such as anti-inflammatory, immunomodulatory, anti-infective, anti-hyperglycemic, and anti-tumor activity against various cancer cells. The main active ingredient in *Andrographis paniculata* (AP) is Andrographolide (AG), a natural diterpenoid with a molecular formula of C20H30O5 [3]. This compound is known to have anticancer effects involving apoptosis, cell cycle, and anti-angiogenic activities. Thus, it has been extensively studied in various cancer cell lines as a chemotherapeutic agent. Its cytotoxic effects are attributed to the lactone moiety, hydroxyl group at C-14, and olefinic bond at Δ12, 13. AG can be found in all parts of AP but is most abundant in the leaves [4]. AG targets many proteins involved in various pathways, so the combination of standard therapy and AG is thought to help resolve resistance issues and improve the effectiveness of ovarian cancer therapy.

The main bioactive components in AP are diterpenoids, flavonoids, and polyphenols. AG, one kind of diterpenoid, is known to have anticancer effects through various signaling pathways. Several pathways involved in AG's anticancer effect include apoptosis, cell cycle arrest, and anti-angiogenesis. Therasa *et al.* found anticancer properties of AP extract by the decrease in Hep-2 human laryngeal cancer cell viability. The study showed DNA fragmentation caused by the degradation of chromosomal DNA after the cells were exposed to AP. On running electrophoresis, Hep-2 exposed to AP extract cells were smeared, which indicated necrosis [5]. In Mao *et al.*'s study, it was found that AG increases Hep-2 sensitivity to carboplatin through apoptotic pathways and by increasing intracellular reactive oxygen species (ROS), which regulates PI3K/AKT pathways and alters the BCL2/BAX ratio, which in turn activates the cleavage of Caspase 3 [6]. AG is also found to inhibit angiogenesis through inhibition of miR-21-5p, which then promotes angiogenesis through TIMP3 [7]. It has also been found to induce cell cycle arrest at the S/G2M or G0/G1 phase [8]. The implications of these findings lead to the assumption that phytopharmaceuticals can

aid in enhancing standardized ovarian cancer treatment with platinum-based chemotherapy [6]. A standard that is known for its resistance and many side effects, which makes it crucial to find an alternative to heighten its medicinal effects while attenuating adverse effects. However, the abundance of other main bioactive components in AP raises whether using AP extract is better than using AG alone. This study examined the combined effects of AG, AP extract, and cisplatin chemotherapy on apoptosis pathways, cell cycle, and angiogenesis in the SKOV-3 human ovarian cancer cell line. This study aims to determine the additive cytotoxic effects of AP and cisplatin on apoptosis pathways (Bax, Caspase-3, Caspase-9, Bcl2), cell cycle (cyclin D), and angiogenesis (VEGF).

## MATERIALS AND METHODS

### Materials

AP ethanol extract was provided by PT. Konimex (Jakarta, Indonesia), following the manufacturer's procedures. Using 90% ethanol as the solvent, the maceration method was used to extract the AP simplicia. For every kilogram of simplicia, a total of 10 l of ethanol was needed. A thick extract was produced by vacuum-evaporating the extract at a temperature of 60 °C until it achieved its maximum thickness, as indicated by the total solid end-point parameter. This method was adopted from Khatimah *et al.*'s study in 2024 [9].

Cisplatin was obtained from MyBioSource. AG was obtained from Plamed, China.

### Cell culture

Low passageSKOV3 cells line under 10th were used in this study were cryopreserved biological materials obtained from the Stem Cells and Tissue Engineering (SCTE) laboratory, IMERI, Faculty of Medicine, Universitas Indonesia. Cells were retrieved from the cryopreservation tank and thawed at 37 °C in a water bath, then seeded into a 96-well plate. The cells were cultured using a complete medium consisting of a mixture of 1% antibiotics, 1% antimycotics, 10% fetal bovine serum (FBS), and a high glucose DMEM basal medium. The cells were seeded into a 75 $cm^2$ flask at a density of 5,000 cells/$cm^2$ for post-cryopreservation recovery. Cell cultures were grown in an incubator at 37 °C with 5% CO2. The medium was replaced every 2 to 3 days until the cells were ready for harvest. Cell harvesting was performed by adding trypsin enzyme to the cell culture. The cells were incubated with the trypsin enzyme for 3 to 5 min in the incubator at 37 °C with 5% CO2. After incubation, the cells were observed under a microscope. Once the cells appeared detached from the culture vessel, the cell suspension was supplemented with a complete medium and centrifuged at 650 g for 5 min. The cell pellet was counted using trypan blue under a microscope. The protocol of this study was approved by the Health Research Ethics Committee University of Indonesia and Cipto Mangunkusumo Hospital, Indonesia (No. KET/148/UN2. F1/ETIK/PPM.00.02/2023).

### CC50 determination

The calculation of the CC50 dose was performed on an ethanol extract of AP, AG and cisplatin. SKOV3 cells seeded in a 96-well plate at a density of 10,000 cells/$cm^2$ were incubated in an incubator at 37 °C with 5% CO2 for 24 h. After 24 h, the cells showed 70% confluency and were ready for treatment. The AP and AG extract solution were diluted in DMSO to create 6 different concentrations: 1, 5, 10, 50, 500, and 1000 (mcg/ml). Cisplatin extract solution was diluted in DMSO to create 6 different concentrations: 10, 15, 20, 25, 30, 35 (mcg/ml).

These six concentrations of these extracts were added to the cells and incubated for 24 h. After 24 h, the cells were treated with MTT reagent (CyQUANT™ MTT Assay) to measure their viability using a spectrophotometer. In the calculation of cell viability, SKOV3 cells were seeded in a 6-well plate at a density of 5,000 cells/cm2 and cultured in an incubator at 37 °C with 5% CO2.

The CC50 doses for each of these extracts in sequence are AP (14 µg/ml); cisplatin (35 µg/ml); and AG (184 µg/ml).

### Drug treatment

After reaching 70% confluency, the cells were treated and incubated for 24 h. The cells were divided into 6 experiment groups: 1) Untreated group; 2) SKOV3 group treated with CC50 cisplatin (35µg/ml); 3) SKOV3 group treated with CC50 cisplatin (35 µg/ml) and CC50 AG (184 µg/ml); 4) SKOV3 group treated with CC50 cisplatin (35 µg/ml) and ½ CC50 AP extract (7 µg/ml); 5) SKOV3 group treated with CC50 cisplatin (35 µg/ml) and CC50 AP extract (14 µg/ml); 6) SKOV3 group treated with CC50 cisplatin (35 µg/ml) and 1.5 CC50 AP extract (21 µg/ml). The cells were then harvested, observed, and counted using trypan blue under a microscope, and RNA isolation was performed for gene expression analysis

### qRT-PCR

RNA isolation from cultured cells was performed using the Quick-RNA Miniprep Kit (Zymo Research). The concentration and purity of RNA were determined using a spectrophotometer. The isolated RNA was then synthesized into cDNA using the ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo). Gene expression analysis with qRT-PCR in this study was conducted using the Applied Biosystems® 7500 Fast machine and SensiFAST™ SYBR® Lo-ROX (Bioline) reagents. Primers used in this study were specifically designed using IDT PrimerQuest software. The sequence of markers that we examined can be seen in table 1.

**Table 1: The sequence of apoptotic, anti-angiogenesis and cycle cell markers that had been examined**

| Gene | Sequence |
|---|---|
| Bax R | CGG CCC CAG TTG AAG TTG |
| Bax F | GAT GCG TCC ACC AAG AAG CT |
| BCL2 R | AGG ACC AGG CCT CCA AGC T |
| BCL2 F | TCC GCA TCA GGA AGG CTA GA |
| CASPS9 R | GCA CCA CTG GGG GTA AGG TTT TCT AG |
| CASPS9 F | ATG GAC GAA GCG GAT CGG CGG CTC C |
| Caspsase 3 R | CAA GCT TGT CGG CAT ACT GTT TCA G |
| Caspsase 3 F | TTC AGA GGG GAT CGT TGT AGA AGT C |
| VEGF R | AGTCTCTCATCTCCTCCTCTTC |
| VEGF F | TGGTGTCTTCACTGGATGTATTT |
| Cyclin D R | AGACGCCTCCTTTGTGTTAAT |
| Cyclin D F | GGGTTGTGCTACAGATGATAGAG |

The output from the PCR machine was in the form of CT values. These values were obtained from the cycle point when the amplification curve entered the exponential phase. The CT values obtained were then processed using the Livak Formula to determine the relative expression value of the target gene to the reference gene (GAPDH). The Livak Formula used in this study is as follows:

$$\Delta CT \text{ sample (day 2, 4, 7, 14)} = CT \text{ target gene} - CT \text{ reference gene}$$

$$\Delta CT \text{ calibrator (day 0)} = CT \text{ target gene} - CT \text{ reference gene}$$

$$\Delta\Delta CT = \Delta CT - \Delta CT \text{ calibrator}$$

$$\text{Relative gene expression} = 2^{(-\Delta\Delta CT)}$$

The data results were presented as mean±SD (standard deviation) using IBM SPSS Statistics 22 software for testing normality and data homogeneity. This was followed by one-way ANOVA with Dunnett's multiple comparisons test as the post-hoc test. Data was considered statistically significant if *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

## RESULTS

### The combination of AG and AP extract with standard therapy can reduce SKOV3 cell viability

We observed the role of combining AG and AP extract with standard therapy on SKOV3 ovarian cancer cells through MTT assay. The results of this study indicated that AG and AP extract had a significant inhibitory effect on SKOV3 cancer cells, as shown in fig. 1. Cell viability inhibition is statistically significant (P<0.0001) in all treatment groups when compared to the control group. The data showed that the mean viability of SKOV3 cancer cells decreased with standard therapy (54%) compared to the control. This mean value

continued to decrease in the treatment groups given AG (37%) and experienced the most significant decrease in the treatment group given a combination of standard therapy and AP extract with sequential results in the groups receiving doses of ½ CC50 AP extract, CC50 AP extract, 1.5 CC50 AP extract at 30%, 23%, 20% respectively. Thus, it can be concluded that AP extract had a better inhibitory effect on SKOV3 cells and is dose-dependent compared to AG (fig. 1A-B).

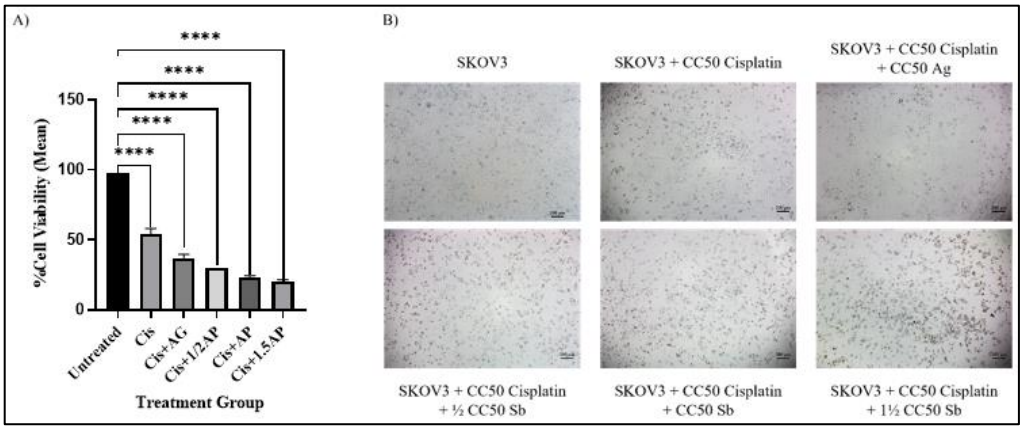


**Fig. 1: *In vitro* activity of AG and AP extract in different concentrations toward SKOV3 cancer cells. n=9 A) the significant effect of AG and AP extract in different concentrations on SKOV3 cell viability was assessed by an MTT assay. The graphic showed that the effect of AP extract on cell viability is dose-dependent. The results were represented as mean±SD (****P<0.0001 compared to the control) B) Morphology appearance of control and treated group on SKOV3 cell. The pointed cells show that the cells are alive, while round cells indicate that the cells have died. The cell density in each group progressively decreased with the treatment administration consisting of standard therapy with AG or increasing doses of AP extract in each group**

### AG and AP extract induced apoptosis in SKOV3 cancer cell

To assess the effects of AG and various doses of AP extract on the apoptosis of SKOV3 cancer cells, we conducted an analysis of pro-apoptosis gene (Bax) and anti-apoptosis gene (Bcl2) expression using qRT-PCR. We found that administering AG and AP extract promoted Bax expression and reduced Bcl-2 levels, shown in fig. 2A and B, respectively. There is a statistically significant increase of BAX expression in the group combined with ½ CC50 AP (P<0.001) and CC50 AP (P<0.01), the best result being the former. BCL2 expression showed a significant decrease in all groups except one treated with cisplatin alone, indicating a synergistic effect in the apoptotic pathway. The group exhibiting the best induction of apoptosis, marked by the highest up-regulation of Bax and the highest down-regulation of Bcl-2, indicating the best apoptosis activity, was found in the group consisted of SKOV3 cells treated with CC50 cisplatin and ½ CC50 AP extract. These results indicated that AG and AP extract could promote apoptosis in SKOV3 cells (fig. 2A-B).

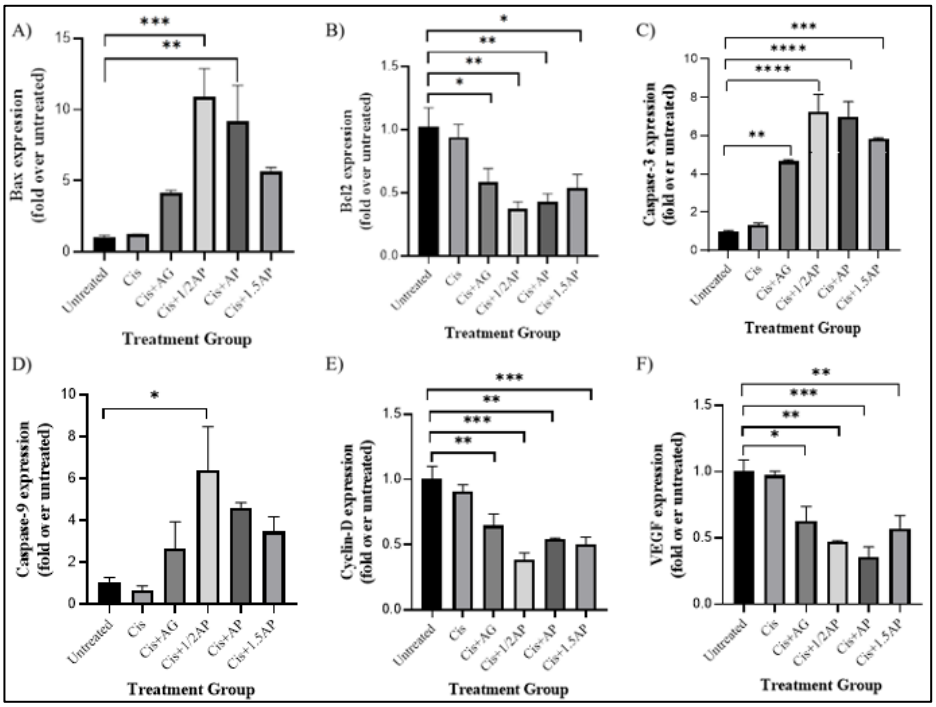


**Fig. 2: Effects of AG and AP extracts in various experiment groups to levels of A) Bax; B) Bcl-2; C) Caspase-3; D) Caspase-9; E)Cyclin D; F) VEGF. n=9, After incubating the cells for 24 h, they were cultured, and RNA isolation was performed to analyze their gene expression using qRT-PCR. The data were presented as mean±SD for all parameters except for caspase-9, which was presented as median and interquartile range (IQR).*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared with the control**

**AG and AP extract increased the activation of caspase 3 and 9 in SKOV3 cancer cells.**

As shown in fig. 3, the administration of AG and AP extract increased caspase 3 and 9 levels, which were a signaling pathway in apoptotic activity, through increasing ROS that triggers the apoptotic cascade. In fig. 2C, caspase 3 increased significantly in all groups where CC50 cisplatin was in combination with CC50 AG (P<0.01) and all concentrations of AP (P<0.0001 for ½ CC50 AP and CC50 AP; P<0.001 for 1.5CC50 AP). In fig. 2D, caspase 9 increased significantly only in the group where CC50 cisplatin is combined with ½ CC50 AP (P<0.05). In these two figures, it is also seen that the therapy standard (cisplatin-only group) slightly increased the level of caspase3 but not caspase9. On the other hand, the additional administration of AG alone could increase both expressions of caspase 3 and 9. However, the highest expression levels of caspase 3 and 9 were found in the treatment group of AP extract, precisely in the group consisting of SKOV3 cells treated with CC50 cisplatin and ½ CC50 AP extract.

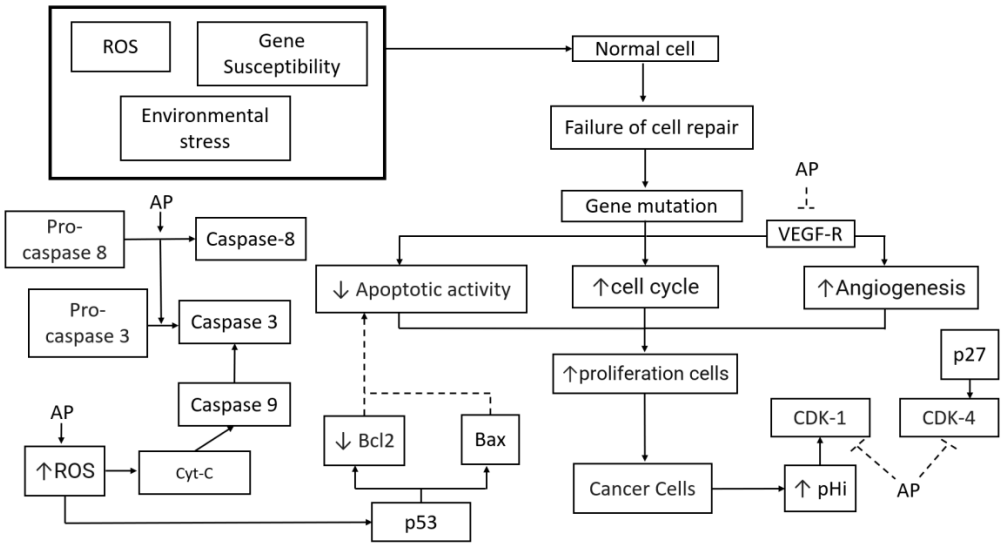


**Fig. 3: Proposed mechanism of AP extract on SKOV3 cells as a potential cytotoxic agent**

**AG and AP extract induced cell cycle arrest in SKOV3 cancer cells**

Cyclin-D is an important mediator that regulate the cell cycle process. Fig. 2E showed a statistically significant decrease in Cyclin-D expression in all groups (P<0.01 for combination with AG and CC50 AP; P<0.001 for ½ CC50 AP and 1.5 CC50 AP) except the group treated with only cisplatin; however, it showed a slight decrease as well. This indicated an additive effect of AP and AG in combination with cisplatin in inducing cell cycle arrest. The results demonstrated that administering AG and AP extract could decrease cyclin-D levels compared to the control, with the most significant reduction found in the SKOV3 group treated with CC50 cisplatin and ½ CC50 AP extract.

**AG and AP extract decreased VEGF levels in SKOV-3 cancer cells**

VEGF expression is an angiogenesis marker. Therefore, increasing its expression was believed to be one factor in promoting tumor growth. Fig. 2F showed a statistically significant decrease in VEGF expression in all groups (P<0.05 for Cis+AG; P<0.01 for Cis+½ CC50 AP and 1.5 CC 50 AP; P<0.001 for Cis+AP) except the cells that are treated with cisplatin alone. From the graphic, the administration of cisplatin alone still caused a slight decrease in the VEGF level compared with the control. There is a more significant reduction in VEGF expression occurring in the combination of cisplatin and AP extract, compared to the combination with AG. The lowest expression of VEGF is seen in the SKOV3 group treated with CC50 cisplatin and CC50 AP extract. This indicated that AG and AP extract had an inhibitory effect on VEGF in SKOV3 cells (fig. 2F).

**DISCUSSION**

A combination of cisplatin and AG or AP extract can promote apoptosis, anti-angiogenesis activity and cycle cell arrest but those properties were better shown in the AP extract group combination. This study found that AG and AP extract possess anti-proliferative properties against SKOV3 cells. The MTT assay results successfully demonstrated that AG and AP extract can significantly decrease the viability of SKOV3 ovarian cancer cells in a dose-dependent manner (fig. 1A). The decrease in the mean viability count in the SKOV3 group treated with cisplatin and ½ CC50 AP and in the SKOV3 group treated with cisplatin and CC50 AP did not show a significant difference. This phenomenon might be because that the ½ CC50 AP dose had already fully occupied the receptors of the SKOV3 cells, so increasing the dose did not significantly further reduce the mean viability count.

In this study, the IC50 dose of AP extract, where the viability of SKOV3 cells decreased to 50%, was found to be at a concentration of 14 µg/ml. Other research using AP extract with different cell lines has reported varying IC50 values. Rajeswari *et al.*, 2022, found the IC50 of AP seed extract to be 103.3 µg/ml against the HepG2 cell line [10]. Research by Rajesh Kumar *et al.*, 2015, showed IC50 of AP leaf extract against H-29 colon cancer cells is at 200µg/ml [11]. Both studies showed much higher IC50 concentration compared to the findings in this study. Variations in IC50 values were also observed in HCT116 and A549 cells, where the IC50 of AP extract was found to be 45.32±0.86 µg/ml and 97.467±1.496 µg/ml, respectively [12].

The variation in IC50 of AP extract across different cell lines may be due to differences in receptor expression and signaling pathways involved in each cell line, which originate from various organs and thus respond differently to AG. Each cell has specific characteristics, such as receptor expression, metabolic pathways, and intracellular conditions. These differences affect the interaction between AG and the cells. In lymphoma cells (MCF-7), prostate cancer cells (PC-3), gastric cancer cells (AGS, SNU601, SUN638), bladder cancer cells (T24) and kidney cancer cells (786-0, OS-RC-2, ACHN cell) the administration of AG activates TRAIL related apoptosis receptors [13]. Research on AG in prostate cancer cells has shown anticancer properties by inhibiting androgen receptors [14]. Different signaling pathways and receptors are also found in melanoma cancer cell lines, where AG induces apoptosis in insulinoma mediated by TLR4 (Tokll-like receptor 4)/NF-κB signaling pathways [15].

Previous studies on other cancer cell lines have also shown similar results, where they found that AG and its derivatives can decrease cell proliferation/viability through different mechanisms depending on the cancer cell line. Generally, the reduction in cancer cell

viability is partly caused by the induction of apoptosis, cell cycle arrest, and inhibition of cancer cell angiogenesis [16]. Research by Chen *et al.*, 2017, found that AG can overcome resistance issues in the ovarian cancer cell lines A2780 cisRandA2780ZD0473R, where it was found that AG activity to kill fifty percent of cancer cell was higher than cisplatin in normal and resistant cancer cell lines. On ovarian cancer cell lines A2780 cisR, the combination of AG and cisplatin also exhibited a strong synergistic effect with combination indices (CI)<1 when cisplatin and AG were administered with a 4-hour time difference, especially at higher concentrations. The greatest synergistic effect was observed when cisplatin was administered first, followed by AG4 or 24 h later (0/4 h and 0/24 h sequence) [17].

Based on microscopic examination, cell morphology in all groups showed two types: pointed cells and round cells (fig. 1B). Pointed cells indicate that the cells are alive, while round cells indicate that the cells have died. Among the treatment groups, we observed that the cell density decreased when standard therapy was administered along with AG or AP extract, with the lowest density found in the SKOV3 group treated with CC50 cisplatin and 1.5 CC50 AP extract. The reduction in density was caused by numerous dead cells detaching from the culture vessel and floating, concurrent with an increase in the dose of AP extract.

From this study, we successfully proved that administering AG and AP extract with standard therapy to SKOV3 ovarian cancer cell lines promotes the apoptosis process by increasing the levels of Bax, initiator and executioner caspases (caspase 9 and caspase 3), while also decreasing the level of Bcl2 (fig. 2A-D). Apoptosis occurs involving various signaling pathways, such as the extrinsic death receptor ligand or the intrinsic mitochondrial pathway that can activate both caspase-dependent and caspase-independent pathways. The Bcl-2 family proteins, including antiapoptotic (Bcl-2) and proapoptosis (Bax) members, play a crucial role in regulating apoptosis via the mitochondrial pathway. Caspase is a protease that functions as an inactive proenzyme in the cytosol. When apoptosis stimuli are present, initiator caspases (caspase 8 and 9) undergo activation to subsequently activate executioner caspases (caspase-3 and 7) [18]. Caspase-3 is a key executioner caspase in apoptosis involved in cleaving many cellular proteins [19].

In MDA-MB-231 breast cancer cells, AGhas been shown to promote apoptosis through induction of mitochondrial cytochrome c and increased expression of Bax, as well as decreased expression of Bcl-2 [8]. Research by Mao *et al.*, 2019, on Hep-2 human laryngeal cancer cells also showed similar results, where administration of AG led to increased expression of p53, Bax, caspase 3, and decreased expression of the anti-apoptosis gene Bcl-2 [6]. In H1975 cells, AG showed similar results on the above apoptosis markers, which increased Bax expression and decreased Bcl-2 expression, as well as the increase of cleaved caspase 3 and cleaved caspase 9, which are pro-apoptotic-related proteins [20]. In human ovarian teratocarcinoma (PA-1), the apoptosis process cells are induced by increased activated caspase-3 and decreased levels of Bcl-2 after administration of AG compared to control [21].

Other proposed mechanisms of how AP impacts the apoptotic pathway involve the interaction of AG to survivin. Survivin is a protein that inhibits apoptosis by blocking the activation of caspase 9 and caspase 3. This protein is highly found in cancer. Survivin works by preventing apoptosome formation, which inhibits procaspase 9 activation and thus decreases caspase 9 and caspase 3 activation [22]. In Wanandi *et al.*, 2020, study, through molecular docking simulation; it showed that AG reduces the affinity for the protein-protein interaction between survivin, caspase 3 and caspase 9. This in turn would increase the activation of caspase 9 and caspase 3, promoting apoptotic activity [22]. The results of this study support this theory as the increase of caspase 3 and 9 is seen in the group where cisplatin is combined with AG and AP extract, with the best seen in combination with ½ CC50 AP. This implies further mechanism that supports apoptosis present in AP, compared to just AG, that might be beneficial to further study.

In addition to apoptosis mechanisms, the decrease in cancer cell viability can also occur through cell cycle arrest mechanisms. This research indicates that the administration of AG and AP extract reduces the levels of cyclin D (fig. 2E). Cyclin D is an essential regulator in the cell cycle that functions in the nucleus. Cyclin D regulates the transition from the G1 to the S phase by regulating cyclin-dependent kinase 4 (CDK) and CDK6. The Cyclin D1/CDK4 complex translocates to the nucleus and phosphorylates the retinoblastoma (RB) protein. Together with Cyclin E/CDK2, it causes the loss of repressive function of the E2F transcription factor, which regulates the transcription of genes specific to cell proliferation. High expression levels of cyclin D promote cell proliferation and tumor growth [23].

AG is a substance that has been studied to cause cell cycle arrest in the G2/M phase in glioblastoma and melanoma cells [24, 26]. This occurs due to increased expression of cell cycle inhibitory proteins such as p38 [24, 25] and p53 [26, 27], which lead to a decrease in cyclin expression necessary for the transition of G to S phase, thus inhibiting the cell cycle process. The study by Khan *et al.* found that administration of low doses of AG (16-128 µM) caused cell cycle arrest in the G2/M phase, while high doses (256 µM) caused cell cycle arrest in the G0/G1 phase [28]. Dai *et al.*, who studied the effects of AG administration on the cell cycle in the SGC7901 gastric cancer cell line, also showed consistent results, where high and medium doses of AG (40 and 20 µg/ml, respectively) induced cell cycle arrest at the G2/M phase [29]. Rajeswari *et al.*, 2022, found similar results in that the majority of Hepg-2 human laryngeal carcinoma cells experience cell cycle arrest in the G2/M phase after the administration of AG [30]. The phase at which cell cycle arrest induced by AG occurs may vary. In HCT-116 colorectal cancer cell line and MCF-7 breast cancer cell line, derivatives of AG arrest the cell cycle in the G1/S phase [31]. Whilst HuCCA-1 bile duct cancer cells have an increased number of cells found and arrested at the G0/G1 phase [32]. In rheumatoid arthritis, AG demonstrates cell cycle arrest in the G0/G1 phase by inhibiting p21 and p27, reducing the Bcl2/Bax ratio, and decreasing the levels of CDK-4 protein [33]. Research by Bhat *et al.*, 2021, shows different results regarding the role of AG in human ovarian teratocarcinoma, where this study found higher cell levels in the G2/M phase in the group given AG compared to the control [21].

Lastly, the results of this research demonstrate the role of AG and AP extract in the process of angiogenesis (fig. 2F), where in line with previous research findings. Peng *et al.*, 2015, studied the effect of AG on VEGF by administering two doses of AG (5, 10 and 25 µM) for 24 h on HK1 and CNE-1 nasopharyngeal cancer cell lines and found that AG exhibited a dose-dependent inhibitory effect on VEGF levels in these cancer cell lines [34]. Similar results were also found by Shi *et al.*, 2017, where VEGF levels decreased after administering AG in hepatoma Hep3B and HepG2 cells [35]. Another *in vivo* study by Nugrahaningsih *et al.*, which examined VEGF expression in 24 C3H mice with mammary adenocarcinoma, found that oral administration of AP extract for 14 d could reduce VEGF expression levels, with the lowest VEGF expression found at a dose of 15 mg/d of AP extract [36]. This study successfully proves that AG and AP extract can decrease the levels of VEGF. AG is a molecule that can bind to the ATP-binding pocket of the VEGF receptor (VEGFR2). VEGFR2 is the primary receptor of VEGF that plays a crucial role in angiogenesis. By binding VEGF to this receptor, VEGF mediates endothelial invasion, cell migration, proliferation, and survival, increases vascular permeability, and promotes neovascularization [37].

*In vivo* studies showed promising apoptotic and anti-angiogenesis effects of AP and cisplatin combination therapy. Rajendrakumar *et al.* studied the nephroprotective properties of AP. In his study, he divided male wistar rats into five groups, one being negative control, one positive control where nephrotoxicity was induced by a single dose of cisplatin 7.5 mg/kg intraperitoneally injected, the third group had only 500 mg/kg of AP, the fourth group had the same dose of AP 15 d before the injection of cisplatin, and the last group had the AP dose with the cisplatin injection. The histopathology results revealed tubular vessel congestion, vacuolar degeneration, renal cell apoptosis, necrosis, and inflammatory cell infiltration indicative of nephrotoxicity. The groups with exposure to AP all had improvements in their histopathology compared to the second group [38]. The same result is shown in Adeoye *et al.*'s study, where

it is supported by other data presenting AP mitigating CP-induced changes in kidney tissue indicated by the decrease of serum creatinine, oxidative stress and urine protein [39].

AP or AG are known to synergize with other chemotherapy agents. Sukardiman*et al.* found that the administration of ethyl acetate fraction of AP combined with doxorubicin significantly increases apoptosis of fibrosarcoma cells in mice [39]. The same study also showed a significant decrease in VEGF protein expression in mice injected with the AP and doxorubicin–implying a synergistic effect in inhibiting neovascularization, tumor proliferation and tumor growth. Another study by Yuan *et al.* where a xenograft murine model using A549 cell showed mice treated with AG extracted from AP combined with Paclitaxel (PTX) had significantly smaller tumors compared to those without treatment or treated with just AG or PTX [40]. These studies provide insight into the potential beneficial synergy of AP combined with chemotherapy in real-life applications. However, it is important to note that AP has poor oral bioavailability. A possible reason for this limitation is due to its chemical structure as a diterpenoid that limits absorption to systemic circulation due to its low water solubility. This necessitates the need for enhancement of AP using soluble agents and bioenhancers in animal studies or clinical trials [41].

Natural compounds have many phytoconstituents that are beneficial in cancer therapy. These compounds generally impact multiple pathways, including the regulation of cytokines, pro-inflammatory proteins, transcription factors, etc. It is also well-known that the combination of phytoconstituents with chemotherapy has improved patient outcomes by enhancing cancer cells' efficacy to the drugs [16] For example, paclitaxel, after a series of trials and error, has finally been approved by FDA in December 1992 as a treatment in refractory ovarian cancer [42] This innovation continues to improve as new technology was found, the use of albumin-bound nanoparticle (nab) enables paclitaxel to avoid severe toxicity caused by formulation and concentrate in the target tumor. The use of nab-paclitaxel was approved in 2005 for the treatment of metastatic breast cancer. The use of AG, a diterpenoid richly found in AP, with methotrexate has been known to decrease the hepatotoxicity of methotrexate when given for arthritis. It reduces TNF-α, IL-1β and IL-6, showing anti-arthritic activity that has better outcomes than a single treatment of methotrexate alone [43].

The main bioactive components in AP are diterpenoids, flavonoids, and polyphenols, where AP contains more than 20 diterpenoids and more than ten flavonoids. AG, a colorless diterpenoid lactone, is known to have anticancer effects through various signaling pathways. Flavonoids isolated from AP exhibit antioxidant properties by capturing free radicals such as ROS or reactive nitrogen species (RNS) [44]. Similarly, polyphenols have antioxidant and anti-inflammatory effects. The abundance of main bioactive components in AP with various benefits was considered a factor that made using AP extract better than AG alone.

## CONCLUSION

In summary, our research revealed that a combination of cisplatin and AG or AP extract can promote apoptosis, anti-angiogenesis activity and cycle cell arrest but those properties were better shown in the AP extract group combination. The proposed mechanism of AP extract on SKOV3 cells is illustrated in fig. 3. The best group that showed the highest level of apoptosis and cell cycle arrest was demonstrated in a group containing CC50 Cisplatin and ½ CC50 AP, while the highest level of anti-angiogenesis properties was seen in group CC50 Cisplatin and CC50 AP extract. Therefore, we can derive two conclusions. First, is that combining cisplatin with AP extract was better than cisplatin and AG in inducing cell cycle arrest, apoptosis, and anti-angiogenesis properties. Second, the combination of cisplatin with AP or AG provides a synergistic effect that enhances the anticancer properties of chemotherapy which might prove beneficial to investigate further for its implications in clinical settings.

## ACKNOWLEDGEMENT

We would like to extend our gratitude to all members of Stem Cell and Tissue Engineering Research Center, Indonesia Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia and Department of Pharmacology and Therapeutics, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia. We would like to extend our gratitude to Revina Tiara Putri and Catherine Belinda Ro for their assistance in the completion of this work.

## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

FVD, N, WA and ML participated in the conceptualization, planning, and data collection of the study. FVD, ML, RTP analyzed the experimental data. N, WA, ML, RTP and CBR assisted FVD in analyzing and interpreting the results. FVD, N, WA, ML, RTP and CBR drafted the manuscript and prepared all the figures. All authors participated in the critical review and modification of the manuscript.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

## REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021 Feb 4;71(3):209-49. doi: 10.3322/caac.21660, PMID 33538338.
2. IARC. Incidence females in 2022. In: Global Cancer Observatory. Geneva: World Health Organization; 2022. Available from: https://gcoiarc.who.int/today/en/dataviz/tables?mode=populationandpopulations=903_904_905_908_909_935andgroup_populations=0andmultiple_populations=1andcancers=25andsexes=2. [Last accessed on 28 Nov 2024].
3. Nisha NC, Sreekumar S, Biju CK. Identification of lead compounds with cobra venom detoxification activity in *Andrographis paniculata* (*Burm. f.*) *Nees* through in silico method. Int J Pharm Pharm Sci. 2016 Jul;8(7):212-7.
4. Warditiani NK, Susanti NM, Arisanti CI, Putri NP, Wirasuta IM. Antidyslipidemia and antioxidant activity of andrographolide compound from Sambiloto (Andrographis paniculata) herb. Int J Pharm Pharm Sci. 2017 Jul 1;9(7):59. doi: 10.22159/ijpps.2017v9i7.18109.
5. Therasa SA, Sobiya G, Parimala SM. Leaves of *Andrographis paniculata* is an antioxidant and anticancer agent. Asian J Pharm Clin Res. 2020 Aug 8;13(8):213-7. doi: 10.22159/ajpcr.2020.v13i8.37014.
6. Mao W, HE P, Wang W, WU X, Wei C. Andrographolide sensitizes hep-2 human laryngeal cancer cells to carboplatin-induced apoptosis by increasing reactive oxygen species levels. Anticancer Drugs. 2019 Aug;30(7):e0774. doi: 10.1097/CAD.0000000000000774, PMID 31306153.
7. Dai J, Lin Y, Duan Y, LI Z, Zhou D, Chen W. Andrographolide inhibits angiogenesis by inhibiting the mir-21-5p/timp3 signaling pathway. Int J Biol Sci. 2017 May 16;13(5):660-8. doi: 10.7150/ijbs.19194, PMID 28539838.
8. Banerjee M, Chattopadhyay S, Choudhuri T, Bera R, Kumar S, Chakraborty B. Cytotoxicity and cell cycle arrest induced by andrographolide lead to programmed cell death of MDA-MB-231 breast cancer cell line. J Biomed Sci. 2016 Apr 16;23(1):40. doi: 10.1186/s12929-016-0257-0, PMID 27084510.
9. Khatimah NG, Arozal W, Barinda AJ, Antarianto RD, Hardiany NS, Shimizu I. *Andrographis paniculata* ethanol extract alleviates high glucose-induced senescence of human umbilical vein endothelial cells via the regulation of mTOR and Sirt1 Pathways. Indones Biomed J. 2024 Aug 28;16(4):333-42. doi: 10.18585/inabj.v16i4.3067.
10. SR, RV, PA. GCMS analysis on *andrographis paniculata* seed extract and its anticancer activity. Int J Appl Pharm. 2022 Jul 28;84-8. doi: 10.22159/ijap.2022.v14ti.5.
11. Rajeshkumar S, Nagalingam M, Ponnanikajamideen M, Vanaja M, Malarkodi C. Anticancer activity of *Andrographis paniculata* leaves extract against neuroblastima (IMR-32) and human colon (HT-29) cancer cell line. World J Pharm Pharm Sci. 2015 May 27;4(6):1667-75.

12. Goel N, Gajbhiye RL, Saha M, Nagendra C, Reddy AM, Ravichandiran V. A comparative assessment of *in vitro* cytotoxic activity and phytochemical profiling of andrographis nallamalayana J. L. Ellis and Andrographis paniculata (Burm. f.) Nees using UPLC-QTOF-MS/MS approach. RSC Adv. 2021 Nov 8;11(57):35918-36. doi: 10.1039/d1ra07496b, PMID 35492784.
13. HU J, LI Y, Xie X, Song Y, Yan W, Luo Y. The therapeutic potential of andrographolide in cancer treatment. Biomed Pharmacother. 2024 Nov;180:117438. doi: 10.1016/j.biopha.2024.117438, PMID 39298908.
14. Liu SH, Lin CH, Liang FP, Chen PF, Kuo CD, Alam MM. Andrographolide downregulates the v-Src and Bcr-Abl oncoproteins and induces Hsp90 cleavage in the ROS-dependent suppression of cancer malignancy. Biochem Pharmacol. 2014;87(2):229-42. doi: 10.1016/j.bcp.2013.10.014, PMID 24161787.
15. Zhang QQ, Ding Y, Lei Y, QI CL, HE XD, Lan T. Andrographolide suppress tumor growth by inhibiting TLR4/NF-KB signaling activation in insulinoma. Int J Biol Sci. 2014;10(4):404-14. doi: 10.7150/ijbs.7723, PMID 24719558.
16. Tundis R, Patra JK, Bonesi M, Das S, Nath R, Das Talukdar A. Anti-cancer agent: the Labdane diterpenoid andrographolide. Plants (Basel). 2023 May 12;12(10):1969. doi: 10.3390/plants12101969, PMID 37653887.
17. Chen S, HU H, Miao S, Zheng J, Xie Z, Zhao H. Anti-tumor effect of cisplatin in human oral squamous cell carcinoma was enhanced by andrographolide via upregulation of phospho-p53 *in vitro* and *in vivo*. Tumour Biol. 2017 May;39(5):1010428317705330. doi: 10.1177/1010428317705330, PMID 28513299.
18. Dehkordi MH, Munn RG, Fearnhead HO. Non-canonical roles of apoptotic caspases in the nervous system. Front Cell Dev Biol. 2022 Feb 23;10:840023. doi: 10.3389/fcell.2022.840023, PMID 35281082.
19. D Arcy MS. Cell death: a review of the major forms of apoptosis necrosis and autophagy. Cell Biol Int. 2019 Apr 25;43(6):582-92. doi: 10.1002/cbin.11137, PMID 30958602.
20. Chen Z, Tang WJ, Zhou YH, Chen ZM, Liu K. Andrographolide inhibits non-small cell lung cancer cell proliferation through the activation of the mitochondrial apoptosis pathway and by reprogramming host glucose metabolism. Ann Transl Med. 2021 Nov;9(22):1701. doi: 10.21037/atm-21-5975, PMID 34988210.
21. Bhat MA, Murthy HN. Isolation of andrographolide from *Andrographis lineata wall*. ex nees var. lawii C.B. clarke and its anticancer activity against human ovarian teratocarcinoma. Pharmacogn J. 2021 May 5;13(3):660-8. doi: 10.5530/pj.2021.13.84.
22. Wanandi SI, Limanto A, Yunita E, Syahrani RA, Louisa M, Wibowo AE. In silico and *in vitro* studies on the anti-cancer activity of andrographolide targeting survivin in human breast cancer stem cells. Plos One. 2020 Nov 19;15(11):e0240020. doi: 10.1371/journal.pone.0240020, PMID 33211707.
23. Montalto FI, DE Amicis F. Cyclin D1 in cancer: a molecular connection for cell cycle control adhesion and invasion in tumor and stroma. Cells. 2020 Dec 9;9(12):2648. doi: 10.3390/cells9122648, PMID 33317149.
24. Liu G, Chu H. Andrographolide inhibits proliferation and induces cell cycle arrest and apoptosis in human melanoma cells. Oncol Lett. 2018 Feb 2;15(4):5301-5. doi: 10.3892/ol.2018.7941, PMID 29552170.
25. Martinez Limon A, Joaquin M, Caballero M, Posas F, DE Nadal E. The P38 pathway: from biology to cancer therapy. Int J Mol Sci. 2020 Mar 11;21(6):1913. doi: 10.3390/ijms21061913, PMID 32168915.
26. Othman NS, Mohd Azman DK. Andrographolide induces g2/m cell cycle arrest and apoptosis in human glioblastoma dbtrg-05mg cell line via erk1/2/c-myc/p53 signaling pathway. Molecules. 2022 Oct 8;27(19):6686. doi: 10.3390/molecules27196686, PMID 36235222.
27. Mehdizadeh R, Madjid Ansari A, Forouzesh F, Shahriari F, Shariatpanahi SP, Salaritabar A. P53 status and G2/M cell cycle arrest are determining factors in cell death induction mediated by Elf EMF in glioblastoma. Sci Rep. 2023 Jul 5;13(1):10845. doi: 10.1038/s41598-023-38021-z, PMID 37407632.
28. Khan I, Khan F, Farooqui A, Ansari IA. Andrographolide exhibits anticancer potential against human colon cancer cells by inducing cell cycle arrest and programmed cell death via augmentation of intracellular reactive oxygen species level. Nutr Cancer. 2018 May 21;70(5):787-803. doi: 10.1080/01635581.2018.1470649, PMID 29781715.
29. Dai L, Wang G, Pan W. Andrographolide inhibits proliferation and metastasis of SGC7901 gastric cancer cells. Bio Med Res Int. 2017 Jan 18;2017:6242103. doi: 10.1155/2017/6242103, PMID 28194420.
30. Rajeswari S, Vidya R. Antiproliferative activity of *Andrographis paniculata* seed extracts on hepatocellular carcinoma cells. Int J Health Sci. 2022 Aug 1;6 Suppl 4:11848-59. doi: 10.53730/ijhs.v6nS4.11409.
31. Wong CC, Lim SH, Sagineedu SR, Lajis NH, Stanslas J. Srj09 a promising anticancer drug lead: elucidation of mechanisms of antiproliferative and apoptogenic effects and assessment of *in vivo* antitumor efficacy. Pharmacol Res. 2016 May;107:66-78. doi: 10.1016/j.phrs.2016.02.024, PMID 26940565.
32. Suriyo T, Pholphana N, Rangkadilok N, Thiantanawat A, Watcharasit P, Satayavivad J. *Andrographis paniculata* extracts and major constituent diterpenoids inhibit growth of intrahepatic cholangiocarcinoma cells by inducing cell cycle arrest and apoptosis. Planta Med. 2014 Apr 29;80(7):533-43. doi: 10.1055/s-0034-1368399, PMID 24782229.
33. Islam MT, Ali ES, Uddin SJ, Islam MdA, Shaw S, Khan IN. Andrographolide a diterpene lactone from *Andrographis paniculata* and its therapeutic promises in cancer. Cancer Lett. 2018 Apr;420:129-45. doi: 10.1016/j.canlet.2018.01.074, PMID 29408515.
34. Peng T, HU M, WU TT, Zhang C, Chen Z, Huang S. Andrographolide suppresses proliferation of nasopharyngeal carcinoma cells via attenuating nf-κB pathway. Bio Med Res Int. 2015;2015:735056. doi: 10.1155/2015/735056, PMID 25861643.
35. Shi L, Zhang G, Zheng Z, LU B, JI L. Andrographolide reduced VEGFA expression in hepatoma cancer cells by inactivating HIF-1α: the involvement of JNK and MTA1/HDCA. Chem Biol Interact. 2017 Aug;273:228-36. doi: 10.1016/j.cbi.2017.06.024, PMID 28651835.
36. Nugrahaningsih N, Sarjadi S, Dharmana E, Subagio HW. VEGF expression of adenocarcinoma mammae after oral administration of *Andrograpahis paniculata* extract. Indonesian J Pharm Sci. 2015 Apr;13(1):29-34.
37. Kajal K, Panda AK, Bhat J, Chakraborty D, Bose S, Bhattacharjee P. Andrographolide binds to ATP binding pocket of VEGFR2 to impede VEGFA-mediated tumor angiogenesis. Sci Rep. 2019 Mar 11;9(1):4073. doi: 10.1038/s41598-019-40626-2, PMID 30858542.
38. Rajendrakumar T, Rao S, Satyanarayana M, Narayanaswamy H, Byregowda S. Effect of *Andrographis paniculata* on cisplatin-induced renal histopathology in wistar albino rats. J Entomol Zool Stud. 2020;8(3):589-96.
39. Adeoye BO, Oyagbemi AA, Asenuga ER, Omobowale TO, Adedapo AA. The ethanol leaf extract of *Andrographis paniculata* blunts acute renal failure in cisplatin-induced injury in rats through inhibition of KIM-1 and upregulation of Nrf2 pathway. J Basic Clin Physiol Pharmacol. 2018 Nov 30;30(2):205-17. doi: 10.1515/jbcpp-2017-0120, PMID 30500779.
40. Sukardiman S, Balqianur T. The role of ethyl acetate fraction of *andrographis paniculata* and doxorubicin combination toward the increase of apoptosis and decrease of VEGF protein expression of mice fibrosarcoma cells. Int J Pharm Pharm Sci. 2015 Apr;7(4):347-50.
41. Yuan H, Sun B, Gao F, Lan M. Synergistic anticancer effects of andrographolide and paclitaxel against A549 NSCLC cells. Pharm Biol. 2016 May 9;54(11):2629-35. doi: 10.1080/13880209.2016.1176056, PMID 27159496.
42. Songvut P, Boonyarattanasoonthorn T, Nuengchamnong N, Junsai T, Kongratanapasert T, Supannapan K. Enhancing oral bioavailability of andrographolide using solubilizing agents and bioenhancer: comparative pharmacokinetics of andrographis paniculata formulations in beagle dogs. Pharm Biol. 2024 Feb 13;62(1):183-94. doi: 10.1080/13880209.2024.2311201, PMID 38351624.
43. Huang M, LU JJ, Ding J. Natural products in cancer therapy: past present and future. Nat Prod Bioprospect. 2021 Jan 3;11(1):5-13. doi: 10.1007/s13659-020-00293-7, PMID 33389713.
44. Bhattacharya S, Mandal SK, Akhtar MS, Dastider D, Sarkar S, Bose S. Phytochemicals in the treatment of arthritis: current

knowledge. Int J Curr Pharm Sci. 2020 Jul 15;12(4):1-6. doi: 10.22159/ijcpr.2020v12i4.39050.

45. Kurniawan DW, Aini Gumilas NS, Arramel, Hartati ND, Novrial D, Tarwadi. Preparation characterization and toxicity study of *Andrographis paniculata* ethanol extract poly lactic-co-glycolic acid (PLGA) nanoparticles in Raw 264. 7 cells. Int J App Pharm. 2024 Jul 7;16(4):78-83. doi: 10.22159/ijap.2024v16i4.50798.