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Antitumor Activities of *Rauwolfia vomitoria* Extract and Potentiation of Carboplatin Effects Against Ovarian Cancer[☆]

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ABSTRACT

Background: Tumor resistance to platinum-based drugs has been an obstacle to the treatment of ovarian cancer. Extract of the plant *Rauwolfia vomitoria* has long been used by cancer patients. However, there have not been systematic studies of its anticancer activity.

Objective: In an effort to enhance the effectiveness of platinum-based drugs, we investigated the anticancer effect of a *Rauwolfia vomitoria* extract (Rau), both alone and in combination with carboplatin (Cp).

Methods: In vitro cytotoxicity and colony formation were evaluated in several ovarian cancer cell lines. In vivo effects were evaluated in an intraperitoneal ovarian cancer mouse model. The combination of Rau and Cp was assessed using Chou-Talalay's constant ratio design and median effect analysis based on the isobologram principle to determine the combination index values.

Results: Rau decreased cell growth in all 3 tested ovarian cancer cell lines dose dependently and completely inhibited formation of colonies in soft agar. Apoptosis was induced in a time- and dose-dependent manner and was the predominant form of Rau-induced cell death. Synergy of Rau with Cp was detected, with combination index values < 1 and dose reduction index values for Cp ranging from 1.7- to 7-fold. Tumor growth in mice was significantly suppressed by 36% or 66% with Rau treatment alone at a low (20 mg/kg) or a high dose (50 mg/kg), respectively, an effect comparable to that of Cp alone. The volume of ascitic fluid and the number of nonblood cells in ascites were also significantly decreased. Combining Rau with Cp remarkably enhanced the effect of Cp and reduced tumor burden by 87% to 90% and ascites volume by 89% to 97%.

Conclusions: Rau has potent antitumor activity and in combination significantly enhances the effect of Cp against ovarian cancer.

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Introduction

With an estimated 22,280 new cases and 15,500 deaths in the United States in 2012, ovarian cancer causes more death than any other cancer of the female reproductive system.¹ Due to the lack of significant symptoms in the early stages and the absence of effective biomarkers for early detection, ovarian cancer is usually diagnosed in patients at a late stage of the disease.^{2–4} As a result, these patients have a poor prognosis and severely impaired quality of life. Although current primary therapy can improve the 5-year survival

rate, it has not increased the overall rate of cure.^{1,4} This is because $> 70\%$ of patients experience a relapse and develop a resistance to platinum- and taxane-based treatments.^{4,5} Malignant ascites resistant to conventional chemotherapy affect 28% of ovarian cancer patients in their last period of life.⁶ Effective and novel treatment strategies for advanced ovarian cancer are urgently needed.

Numerous studies have attempted to improve the efficacy of standard platinum-based therapy by incorporating newer cytotoxic agents. A promising strategy is to use natural products with anticancer effects in combination with platinum-based drugs. One of the advantages of some natural products is their low toxicity compared with conventional chemotherapy drugs. Combinations of natural compounds with standard chemotherapy drugs may exert additive or synergistic effects on killing cancer cells, thereby allowing lower and safer doses of the more toxic drug to be used.

Herbal preparations of *Rauwolfia vomitoria*, a tropical shrub in the family of Apocynaceae, have been used in traditional folk medicine in Africa to treat a variety of ailments including fever, general weakness, gastrointestinal diseases, liver diseases,

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psychosis, pain, and cancers.^{7–14} Extracts from this plant are enriched in β -carboline alkaloids and indole alkaloids.^{15,16} Many of these alkaloids have been isolated from the stem, leaf, and root of *Rauwolfia vomitoria*. From the root alone, there are mainly 5 types of > 20 alkaloids identified.^{15,17–19} Table 1 listed the main alkaloids isolated from the *Rauwolfia vomitoria* root. Reserpine, a drug to control high blood pressure and relieve psychotic symptoms, was isolated from the root bark of *Rauwolfia vomitoria*.^{20–22} Other reported activities of the isolated compounds mainly affect the neurological and cardiovascular systems, with many of them not studied for their bioactivities.

The anticancer activities of these components have barely been studied. One early study in 1986 suggested anti-lymphoma ascites cells effects of 3 alkaloids, alstonine, serpentine, and sempervirine, in specific conditions.²³ Another study in 2006 reported on the anti-prostate cancer activity of *Rauwolfia vomitoria*, but the active anticancer component in the extract was not known.²⁴ Apart from the investigations of its components, the extract as a whole is widely used and actively studied. The extract of this medical herb as a whole mixture has been a traditional medicine for > 2000 years in Africa for the treatment of hypertension and mental disorders. The effectiveness has been confirmed in more recent studies to be mainly as antipsychotic, antihypertensive, anti-inflammatory and improving blood chemistry. A *Rauwolfia*-citrus tea is in an early phase clinical trial in Denmark for its antidiabetic effect.⁷ In this study, we evaluated an important but not yet well-understood aspect of the effect of an extract from the root of *Rauwolfia vomitoria*, enriched with alkaloids and with reserpine removed, in the treatment of ovarian cancer, used alone and in combination with carboplatin (Cp).

Materials and Methods

Experimental materials, cell lines, and cell viability assay

Human ovarian cancer cell lines OVCAR-5 and OVCAR-8 were obtained from the American Type Culture Collection (Manassas, Virginia), SHIN-3 was donated by Dr. Perter Eck at the National Institutes of Health.²⁵ Immortalized human lung epithelial cells MRC-5 were provided by Dr. Sittampalam at the University of Kansas Medical Center and were compared with cancer cells. All

the cells were cultured at 37°C in 5% CO₂/95% air in recommended growth media containing 10% fetal calf serum.

Rauwolfia vomitoria extract (Rau) was provided by Natural Source International (New York, New York). The extract was prepared from air-dried clean roots of *Rauwolfia vomitoria*, first powdered with an electric blender and then extracted twice with 80% ethanol, according to a reported method.²⁶ From the extract, reserpine was removed by chloroform extraction conducted efficiently in columns. Quality control was ensured by HPLC. All the experiments used the extract from a single lot. Rau and Cp (Sigma, St. Louis, Missouri) were prepared in sterile water and stored at –20°C.

Cells were tested for viability by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at 48 hours of treatment. Cells in the exponential growth phase were exposed to serial dilutions of Rau, Cp, or a combination of the two for 48 hours. The incubation time was determined by pre-experiments to obtain the optimal cell death effect. Then cells were changed to fresh media containing MTT and incubated for 4 hours. The colorimetric MTT assay assessed relative proliferation, based on the ability of living, but not dead, cells to reduce MTT to formazan.^{27,28} Cells did not reach the plateau phase during the incubation period. IC₅₀ was defined as the concentration of drug that inhibited cell growth by 50% relative to the untreated control.

Anchorage-independent colony formation assay

Anchorage-independent colony formation assays in soft agar were used to determine long time survival of tumorigenic cancer cells after treatments. In 6-well plates, SHIN-3 cells (5000 cells per well) were seeded in the upper layer containing 0.5% agar, Dulbecco's modified Eagle medium, and 10% fetal bovine serum, with or without 400 μ g/mL Rau. The solid agar base (lower layer) contained 0.75% agar and complete growth medium, with or without 400 μ g/mL Rau, respectively. After 20 days of incubation, colonies were visualized by crystal violet staining, and the number of colonies was counted.

Apoptosis detection by flow cytometry

Cells were exposed to various concentrations of Rau for 48 hours. Cells were washed in phosphate-buffered saline, resuspended in

Table 1
Major alkaloids and their bioactivities isolated from the root of *Rauwolfia vomitoria*.

Type	Alkaloids Detected	Activities Reported	Reference
Yohimbine	α -Yohimbine	Pre- and postsynaptic α_2 -adrenoceptor inhibitor, stimulant and aphrodisiac effect, antidiabetic	41,42
18-Hydroxy-yohimbine	Reserpine	Antipsychotic, antihypertension, antidepressant, anticancer, carcinogenesis	43–48
	Rescinnamine	Antihypertension	49
Heteroyohimbine	Aricine	Unknown	
	Reserpiline	Antipsychotic, antigastric secretion, antihypertension	50–53
	Isoreserpiline	Antipsychotic	8,53
Dihydroindole	Sarpagine	Unknown	
	Ajmaline	Antiarrhythmia, antifibrillation, cardiac and liver toxicity	54,55
	Sandwicine	Unknown	
	Iso-sandwicine	Unknown	
	Mitoridine	Unknown	
	Rauvomitine	Unknown	
	N-demethyl-rauvomitine	Unknown	
	Purpeline	Unknown	
	Seredamine	Unknown	
	Suaveoline	Unknown	
	Tetraphyllicine	Myocardial excitation	56
	Vomalidine	Unknown	
	Serpentine	Antioxidant, possible anticancer	23,40
	Alstonine	Antipsychotic, possible anticancer	23
Ψ -Indoxyl	Isoreserpiline- Ψ -indoxyl	Unknown	
Oxindole	Carapanaubine	Unknown	
	Rauvoxine	Unknown	

binding buffer, and subjected to fluorescein isothiocyanate-conjugated annexin V and propidium iodide (PI) staining according to the manufacturer's protocol (BD Biosciences, San Jose, California). Cells were analyzed by flow cytometry. Annexin V-positive and annexin V-PI double-positive cells were identified as apoptotic cells, whereas PI single-positive cells were identified as necrotic cells.

Western blot

Forty micrograms of protein were loaded for SDS-PAGE. Primary and secondary antibodies were from Cell Signaling Technology Inc. (Danvers, Massachusetts): rabbit anti-poly (ADP-ribose) polymerase (PARP) (1:2000), rabbit anti-caspase-3 (1:1000), rabbit anti-caspase-8 (1:1000), mouse anti-β-actin (1:1000), and goat anti-rabbit or anti-mouse immunoglobulin G (1:5000). Blots were developed using immobilon chemiluminescent substrate (Thermo Scientific, Waltham, Massachusetts).

Intraperitoneal ovarian cancer mouse model

Nude mice were injected with SHIN-3 cells by intraperitoneal injection (2.6×10^6 /mouse). Seven days after tumor cell inoculation, treatment began with intraperitoneal injection of Cp (15 mg/kg weekly), Rau (20 or 50 mg/kg/d), the respective combination of Cp and Rau, and saline solution as control. After 23 days of treatment, mice were euthanized. All tumor lesions in the peritoneal cavity were collected and weighed; ascites were collected, and nonblood cells were counted in ascitic fluids as an index of tumor cells in the fluids. Major organs such as the liver, kidney, and spleen were fixed in formaldehyde and subjected to histological analysis for any damage caused by potential drug toxicity.

Data analysis

MTT data were normalized to their corresponding untreated controls for each condition (drug, cell type) and were expressed as a percentage of viability. Dose-reduction index (DRI) values for Cp were calculated by the equation $DRI_{ICx} = (D_{Cp}/D_{Cp+Rau})$, where D_{Cp} is the dose of Cp alone required to produce an ICx level of cytotoxicity, and D_{Cp+Rau} is the dose of Cp needed to produce the same ICx level of cytotoxicity when it is combined with Rau (at a given molar ratio). DRI_{Cp} is defined with respect to Cp. Combination index (CI) values were calculated by the equation $CI_{ICx} = (D_{CpCombo ICx}/D_{Cp ICx}) + (D_{RauCombo ICx}/D_{Rau ICx}) + \alpha[(D_{CpCombo ICx})(D_{RauCombo ICx})/(D_{Cp ICx})(D_{Rau ICx})]$, where D is the

dose of Cp and Rau, either alone or in combination, at a given constant ratio required to produce an ICx level of cytotoxicity.^{29–31} The more conservative assumption of mutual exclusivity was adopted ($\alpha = 0$). SPSS version 15.0 (SPSS Inc., Chicago, Illinois) was used for additional statistical analysis.

Results

Inhibition of ovarian cancer cell viability and colony formation by Rau

Human ovarian cancer cell lines (SHIN-3, OVCAR-5, and OVCAR-8) were subjected to Rau treatment to test for sensitivity at concentrations from 0 to 800 μg/mL. An immortalized nontumorigenic epithelial cell line (MRC-5) was subjected to the same treatment for comparison. The dose-response curves showed that all cancer cells were susceptible to Rau treatment with IC₅₀ values of ~300 μg/mL, whereas the immortalized noncancerous cells MRC-5 was less sensitive with IC₅₀ = 566 μg/mL, a significant difference compared with cancer cells (Figure 1A). At a concentration of 400 μg/mL, Rau was able to reduce cell viability by 60% to 80% in all 3 tested ovarian cancer cells and only decreased viability in MRC-5 cells by 28%.

Colony formation in soft agar was used to assess the drug effect on tumor cell survival for a longer time, as well as to assess the survival of tumorigenic cells, which are important for in vivo tumor formation.^{32,33} Untreated SHIN-3 cells formed colonies in soft agar at a rate of 23%. Rau at 400 μg/mL completely inhibited formation of colonies of SHIN-3 cells in soft agar (Figure 1B), indicating no survival of tumorigenic cancer cells with this treatment.

Induction of apoptosis in ovarian cancer cells by Rau

To assess the Rau-induced death pathway, annexin V/PI staining was performed to detect apoptosis versus necrosis in SHIN-3 cells treated with Rau. Data from flow cytometry demonstrated that predominant apoptosis was induced by Rau treatment. With 0, 50, 100, and 400 μg/mL Rau treatment, the percentage of positive cells with annexin V and PI staining were 5.7%, 33.6%, 49.6%, and 85.3% respectively (Figure 2A). The induction of apoptosis was clearly dependent on the concentration of Rau with a logarithmic relationship (Figure 2A) and was the major form of cell death induced by Rau. Necrosis (cells that were PI positive only) contributed only 6% to 14% of total cell death all across the Rau concentrations.

Consistent with this finding, Western blot analysis detected extensive cleavage of caspase-8, caspase-3, and PARP in Rau-treated

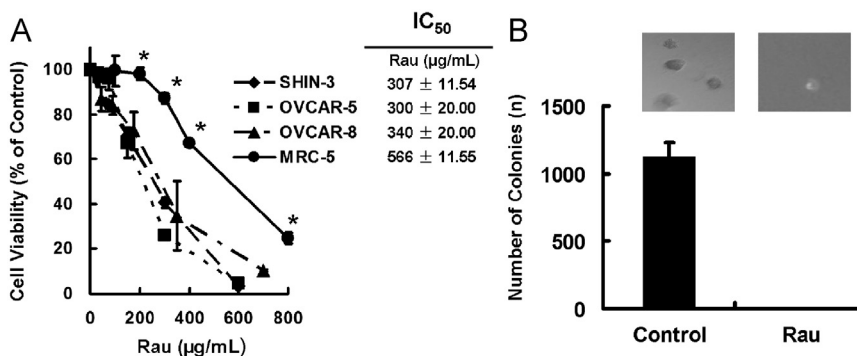


Fig. 1. Cytotoxicity of *Rauwolfia vomitoria* extract (Rau) in normal cells and ovarian cancer cells. (A) Dose-response curves of normal and ovarian cancer cells. Ovarian cancer cells SHIN-3, OVCAR-5, and OVCAR-8 were exposed to serial concentrations of Rau for 48 hours, and cell viabilities were detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. A noncancerous epithelial cell, MCR-5, was subjected to the same treatment. IC₅₀ was defined as the concentration of drug that inhibited cell growth by 50% relative to the untreated control. (*P < 0.05 relative to any of the cancer cells). (B) Colony formation of SHIN-3 cells in soft agar with and without Rau treatment. Five thousand SHIN-3 cells per well in 6-well plates were either treated with 400 μg/mL Rau or untreated (Control). All values are expressed as mean ± SD of 3 independent experiments.

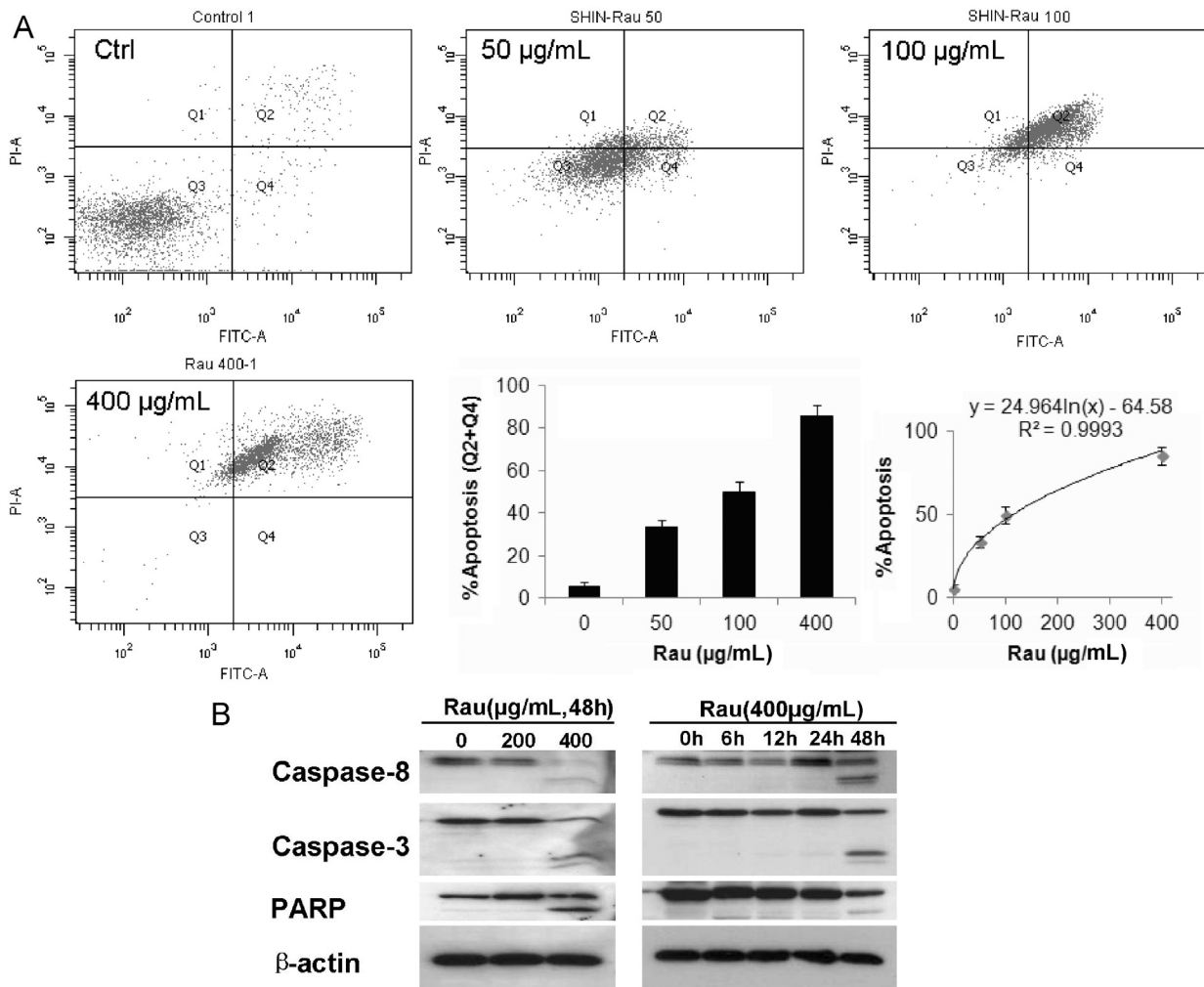


Fig. 2. Apoptosis in SHIN-3 cells induced by *Rauwolfia vomitoria* extract (Rau). **(A)** Flow-cytometry detection of apoptotic cells. SHIN-3 cells were treated with 400 µg/mL of Rau for 48 hours and then underwent double staining with fluorescein isothiocyanate-conjugated annexin V and propidium iodide. The rate of apoptosis was represented by the percentage of cells in the Q2 and Q4 quadrants. A logarithmic relationship between the apoptosis rate and concentration of Rau was shown. **(B)** Cleavage of caspases and poly(ADP-ribose) polymerase (PARP) in SHIN-3 cells treated with Rau. Cells were treated with Rau at the indicated concentrations and time. The dose-dependent and time-dependent cleavage of caspase-8, caspase-3, and PARP were detected by Western blot. β-Actin acted as a loading control.

SHIN-3 cells in a dose-dependent and time-dependent manner (Figure 2B).

Synergy of Rau in combination with Cp against ovarian cancer cells

As platinum-based treatment is a current primary care for ovarian cancer patients, the effect of the combination of Rau and Cp was examined using cultured ovarian cancer cells. After determining the dose-response relationships for Rau (Figure 1A), the dose-response relationships for Cp cytotoxicity were established in SHIN-3, OVCAR-5, and OVCAR-8 cells (Figure 3A, dotted lines). A constant ratio design was used to systematically examine combination dose-response relationships between Cp and Rau. The ratio of Cp to Rau was chosen as $IC_{50Cp}:IC_{50Rau}$. Combination data were presented in terms of Cp concentration. Despite the inherent sensitivity to Cp, results clearly showed that when Rau was added to Cp, decrease in cell viability was dramatically enhanced in all tested cells compared with Cp as a single agent (Figure 3A).

The CI value for each cell type was calculated relying on Chou-Talalay's isobologram principle to determine whether drug combinations were synergistic ($CI < 1$), additive ($CI = 1$), or antagonistic ($CI > 1$).^{28–30} In addition, DRI value for Cp was calculated as described in the Material and Methods section. DRI values > 1 indicate a favorable combination, whereas a DRI value < 1 would be interpreted

as an antagonistic combination. In all cell lines tested, CI values were ≤ 1 (Figure 2B), and DRI values for Cp were > 1 (Figure 2C) across the desired levels of effect (f_a , fraction affected). This indicated an additive to synergistic effect when Rau was combined with Cp. Reduction in Cp doses ranged from 1.7- to 7-fold when Rau was combined with Cp, depending on cell lines and the aimed level of effect. These data unequivocally support the conclusion that the concentration of Cp can be decreased to produce an equitoxic effect on ovarian cancer cells when Rau is combined with Cp.

In vivo tumor inhibitory effect of Rau alone or in combination with Cp

An intraperitoneally implanted SHIN-3 mouse xenograft model was used to evaluate the effect of Rau and the combination of Cp and Rau (Cp + Rau) treatment. Compared with the subcutaneous implantation model, the intraperitoneal model better mimics the clinical condition of advanced human ovarian cancer, especially in peritoneal metastasis and ascites formation. Tumor-bearing mice were treated for 23 days, and necropsy was performed at euthanasia. All tumor lesions in the peritoneal cavity were collected and examined, as was ascitic fluid.

As shown in Figure 4A, Rau treatment alone decreased total tumor weight by 36% and 66% at the daily dose of 20 mg/kg or 50 mg/kg, respectively, compared with saline solution-treated

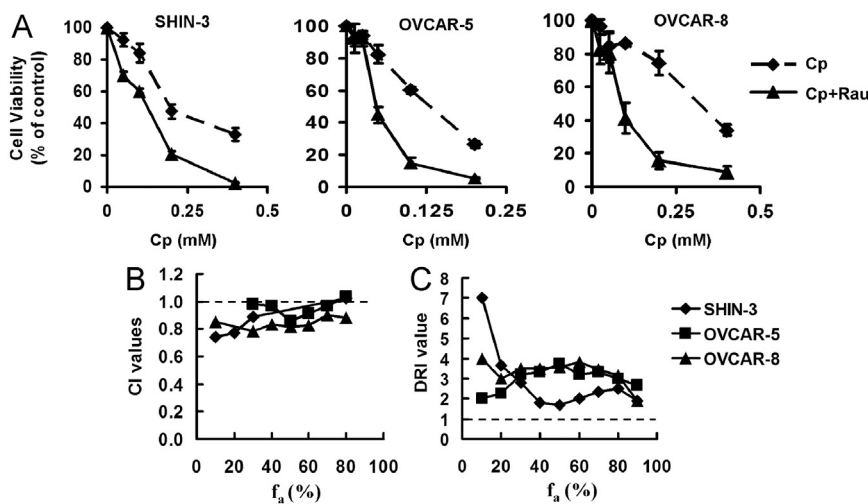


Fig. 3. Combination effect of *Rauwolfia vomitoria* extract (Rau) and carboplatin (Cp) in ovarian cancer cells. (A) Dose-response curves. Ovarian cancer cells were treated with Cp (dotted line) and the combination of Cp and Rau (Cp + Rau, solid line) for 48 hours. The molar ratio of the combination was IC_{50Rau}:IC_{50Cp}. (B) Combination index (CI) across the fraction affected (f_a). The molar ratio of Rau:Cp was IC_{50Rau}:IC_{50Cp}. A CI value > 1 indicates antagonism, a CI value of 1 indicates additive effect, and a CI value < 1 indicates synergy. (C) The dose-reduction index (DRI) across the f_a for Cp when Rau was combined.

control. Cp at the dose used decreased tumor weight by 51%. By combining Rau with Cp, the tumor inhibitory effect was dramatically enhanced. Tumor weight decreased 87% (Cp + 20 mg/kg Rau) and 90% (Cp + 50 mg/kg Rau) relative to control.

The enhancement was significant compared with either Rau or Cp single-agent treatment.

Excessive volumes of ascites were formed in saline solution-treated control mice at the endpoint of the experiment (4 mL of

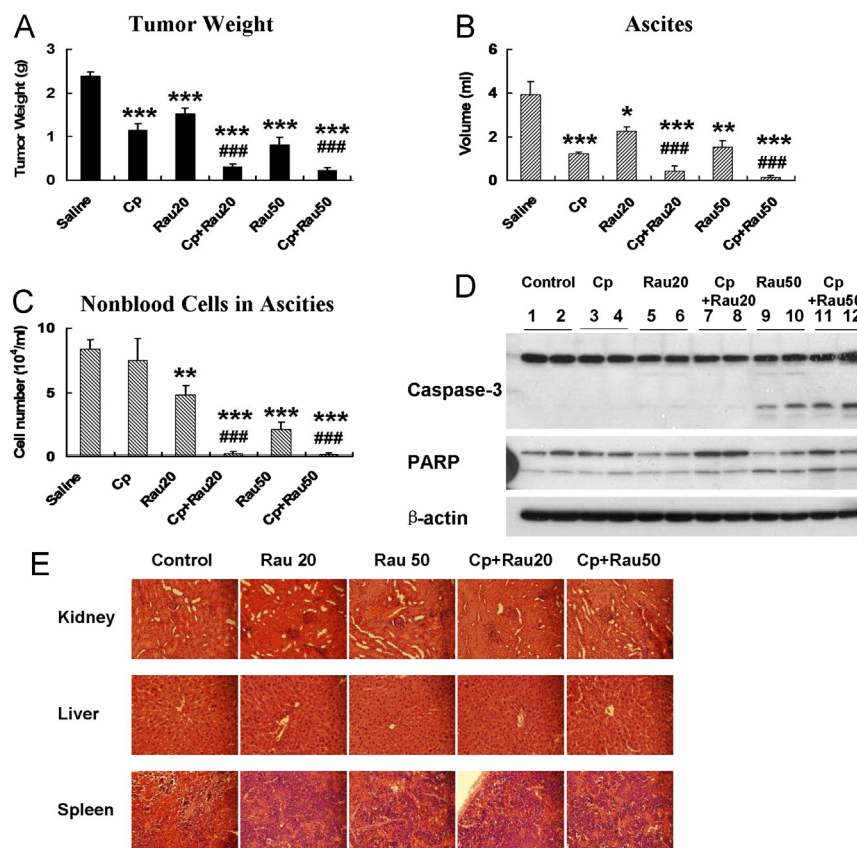


Fig. 4. Effect of *Rauwolfia vomitoria* extract (Rau) and the combination of Rau and carboplatin (Cp) against SHIN-3 ovarian cancer cells in an intraperitoneal mouse model. Cells (2.6×10^6) were intraperitoneally injected into nude mice. After 7 days of tumor inoculation, treatment commenced with an intraperitoneal injection of Rau at 20 mg/kg/d (Rau20) or 50 mg/kg/d (Rau50), Cp at 15 mg/kg/wk, and respective combinations of Rau and Cp (Cp+Rau20, and Cp+Rau50). Treatment lasted for 23 days, and tumor weight, volume of ascites, and number of cells in ascites were assessed at the end of treatment. (A) Total tumor weight. (B) Volume of ascitic fluids. (C) Number of nonblood cells in the ascitic fluids. (D) Cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP) in tumor samples from different treatment groups. (E) Hematoxylin and eosin staining of major organs from different treatment group ($\times 400$ magnification). Kidney, liver, and spleen were collected from each treatment group and fixed in 4% formaldehyde and later underwent histological analysis. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ relative to control group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ relative to Cp-treated group.)

ascites/mouse in average). Rau treatment alone significantly decreased the volume of ascitic fluid to an average of 2.3 mL per mouse at the dose of 20 mg/kg and to 1.5 mL per mouse at the dose of 50 mg/kg ($P < 0.05$). The degree of reduction is comparable to that of Cp (Figure 4B). By combining Rau with Cp (20 or 50 mg/kg Rau), the combination treatments decreased ascites volume by 89% and 97% compared with control to a minimal amount of 0.44 mL per mouse and 0.13 mL per mouse (Figure 4B).

Nonblood cells in ascitic fluid were counted as an estimation of tumor cells in the ascites. The results were shown as the number of cells per milliliters of ascites. Although Cp at the doses used did not reduce the number of cells in ascites, Rau showed a strong effect in decreasing the number of cells per milliliters of ascites by 43% and 75%. Moreover, when combined with Cp, Rau at either of the doses used decreased the number of cells per milliliters of ascites by ~98% (Figure 4C).

Proteins were isolated from tumor samples of the treated and control mice. Western blot analysis showed cleavage of caspase-3 and PARP in Rau and Cp + Rau treatment groups at either high or low doses of Rau (Figure 4D), whereas Cp treatment alone did not cause cleavage in caspases and PARP. These results confirmed the in vitro data that Rau induced apoptosis in tumor cells.

None of the mice demonstrated observable toxicity associated with the treatments. At the end of the experiments, major organs (kidney, liver, and spleen) were underwent hematoxylin and eosin staining and histological analysis. No tissue damage was detected in the treatment groups, and there were no significant differences between control group and treated groups (Figure 4E).

Discussion

Inherent or acquired drug resistance has been a serious problem in ovarian cancer therapy. Combining platinum-based chemotherapy with other anticancer drugs has been an effective way to improve therapeutic outcome.^{34–36} *Rauwolfia vomitoria* has been a folk medicine for centuries, and its extract has been shown to provide antioxidant and antiproliferative effects in different systems based on its inhibition of diverse cellular events associated with tumor pathogenesis.^{23,24,37} Our data demonstrated that Rau substantially inhibited ovarian cancer cell growth, both in vitro and in vivo. Despite the inherent Cp sensitivity of the cell lines, Rau increased chemosensitivity of all tested ovarian cancer cells and synergized with Cp in the inhibition of ovarian cancer.

Importantly, Rau inhibited the tumor growth in a mouse model with intraperitoneal metastasis and massive ascites formation, either alone or in combination with Cp. With Rau and Cp combination treatment, a remarkable tumor inhibition of >90% was achieved, and the ascites were nearly eradicated. This effect was not achieved by Cp treatment alone. It is possible that different degrees of additive/synergistic effects will be achieved if conditions of combination are different, such as the doses of both drugs to be combined, the time and order of administration, and the intervals of treatment. The study here provides a basis for further investigation in refining the combination regimen to achieve optimal effects, even possible complete tumor inhibition. As the prognosis and survival rate are poor for ovarian cancer patients who have intraperitoneal metastasis,¹ these results provided a basis for further investigation for Rau as an adjuvant treatment for ovarian cancer patients.

Moreover, our data showed that Rau had relatively low toxicity on normal cells. This was evident in mice treated with Rau in which major organ toxicities were absent. As many studies have

reported psychological and cardiovascular activities of Rau, the anticipated toxic side effects could be hypotension, cardiac toxicity, psychological effects, and gastrointestinal disturbance. However, in our extract, the compound reserpine was removed, which is the main contributor to the hypotensive effect and part of the psychological effects. Therefore, low toxicity with our extract could be expected. Consistent with this hypothesis, no abnormalities or toxic signs were observed in the mice. In summary, the antitumor activities of Rau synergizing with Cp suggest that this herbal preparation could potentially offer therapeutic benefit in the management of ovarian cancer.

Although potential benefit was suggested by our study, the mechanism(s) of Rau that induced an anticancer effect warrant further study. A previous study reported DNA damage and cell cycle inhibition induced by Rau in prostate cancer cells.²⁴ Activation of these pathways could lead to apoptosis, which is a powerful tumor-suppressive pathway preventing the uncontrolled proliferation of cancerous cells and potentially depleting stemlike and progenitor cancer cell pools.^{38,39} Consistent with these reports, our study showed that Rau mainly induced apoptosis in vitro and in vivo, as shown by flow cytometry and the cleavage of caspase-8, caspase-3, and PARP. The in vitro tumorigenic capacity of ovarian cancer cells was completely inhibited by Rau treatment. However, because this plant preparation contains a complex mixture of natural compounds, there is potential to affect multiple molecular targets and pathways.

Bioactive compounds are often found in medicinal plant and herbal mixtures, making them a superb source for the discovery of novel drug leads. The β -carboline- and indole alkaloid-enriched Rau appears to contain components that possess potent anticancer activity. Our study demonstrated for the first time the anticancer and chemo-potential activity of the mixture of Rau in ovarian cancer, with novelty and significance. Because its anticancer components are largely unknown, it is possible to trace the anticancer activity to single compound, such as alstonine and/or serpentine.^{23,40} On the other hand, there is also the possibility that several components work in concert to exhibit optimal anticancer activity and lower toxicity, such as that seen in many herbal medicines and traditional Chinese medicines. Also, a nonlinear or U-shape dose-response in vivo has been reported by other investigators, further suggesting that different components in the extract were working together.²⁴ Although further studies are needed to investigate these interesting questions, the data presented here provide a basis for identifying the anticancer activity of Rau. Active components could be isolated and developed for optimizing the efficacy, toxicity, and other profiles that could lead to anticancer drug development.

Acknowledgments

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Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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