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Impact of Blood Dragon Resin on Colorectal Cancer Cells through Apoptosis, Autophagy and Cell Cycle Arrest

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Abstract

Introduction: *The rising global prevalence of cancer, coupled with the adverse effects of conventional therapies, underscores the urgent need for safer and more effective treatment options. Natural plant-based remedies have emerged as promising alternatives due to their multi-targeted therapeutic properties. Dracaena cinnabari (DC) resin, commonly known as* Dragon's Blood, has shown potential anti*cancer effects. Thus, this study investigates its cytotoxic and cell death-inducing activities against human colorectal cancer cells.*

Methods: *Methanolic extract of DC was evaluated for its anticancer effects on human colorectal cancer cells (HT-29). Anti-proliferative activity was determined using the Sulforhodamine B (SRB) assay. Flow cytometry was employed to examine the impact of DC on cell cycle progression, as well as its ability to induce apoptosis and autophagy.*

Results: *The methanolic extract of DC resin exhibited significant, concentration-dependent cytotoxicity in HT-29 colorectal cancer cells, with a half maximal inhibitory concentration (IC_{50}) value of 31.82 µg/mL. Flow cytometry analysis revealed that DC induced cell cycle arrest at the S and Sub-G1 phases. Moreover, treatment with DC resulted in a markedly higher proportion of late-stage apoptotic and autophagic cells, with apoptosis reaching 83.21%, compared to the standard chemotherapeutic agent; 5-fluorouracil (5-FU).*

Conclusion: *The findings demonstrate that DC methanolic extract exerts strong anticancer effects through multiple mechanisms, including apoptosis, autophagy, and disruption of cell cycle progression. This highlights its promise as a natural therapeutic agent for colorectal cancer and provides a rationale for further preclinical and in vivo studies to validate its efficacy and safety.*

Keywords: *Colorectal Cancer, Socotra Dragon Blood Tree, Dracaena cinnabari, Anticancer, Autophagy, Apoptosis, Cell Cycles.*

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تأثير رانتنج دم التنين على خلايا القولون والمستقيم من خلال الاستماتة والالتهام الذاتي وتوقف دورة حياة الخلية

أروى أحمد عوض عاطف

الملخص

المقدمة: نظرًا للارتفاع المتزايد في معدلات الإصابة بالسرطان عالميًا، إلى جانب الآثار الجانبية للعلاجات التقليدية، ظهرت الحاجة الملحّة إلى إيجاد خيارات علاجية أكثر أمانًا وفعالية. لقد برزت العلاجات الطبيعية المستمدة من النباتات كبدائل علاجية واعدة نظرًا لخصائصها العلاجية متعددة الأهداف. ويُعرف راتنج نبات *Dracaena cinnabari*، المعروف باسم دم الأخوين (Dragon's Blood)، بإمكاناته المضادة للسرطان. لذلك، هدفت هذه الدراسة إلى تقييم تأثيراته السمية الخلوية وقدرته على تحفيز موت خلايا سرطان القولون والمستقيم.

المنهجية: تم تقييم التأثيرات المضادة للسرطان للمستخلص الميثانولي من راتنج *Dracaena cinnabari* *(DC)* على الخلايا البشرية لسرطان القولون والمستقيم *(HT-29)*. وقد تم تحديد النشاط المضاد للتكاثر باستخدام اختبار *Sulforhodamine B (SRB)*. كما استُخدمت تقنية القياس الخلوي بالتدفق *(Flow Cytometry)* لدراسة تأثير المستخلص على تقدم دورة الخلية بالإضافةً إلى قدرته على تحفيز الاستماتة *(Apoptosis)* والالتهام الذات *(Autophagy)*.

النتائج: أظهر المستخلص الميثانولي لراتنج *DC* تأثيرًا سامًا ملحوظًا يعتمد على تركيز المادة في خلايا سرطان القولون والمستقيم *HT-29*، حيث بلغت قيمة IC_{50} حوالي *31.82* µg/mL. وكشف تحليل القياس الخلوي بالتدفق أن المستخلص تسبب في إيقاف دورة الخلية عند مرحلتي *S* و *Sub-G1*. علاوة على ذلك، أدى العلاج بالمستخلص إلى زيادة كبيرة في نسبة الخلايا التي تمر بمرحلة الاستماتة المتأخرة وكذلك الخلايا التي تخضع لعملية الالتهام الذاتي، حيث وصلت نسبة الاستماتة إلى *83.21%* مقارنةً بالعقار الكيميائي القياسي 5-فلورويوراسيل *(5-FU)*.

الاستنتاج: تُظهر النتائج أن المستخلص الميثانولي لنبات *DC* يمتلك تأثيرات قوية مضادة للسرطان من خلال آليات متعددة، تشمل تحفيز الاستماتة، وتنشيط الالتهام الذاتي، وإحداث اضطراب في تقدم دورة الخلية كما تشير إلى إمكانية استخدامه كعامل علاجي طبيعي واعد لسرطان القولون والمستقيم، مما يستدعي إجراء مزيد من الدراسات قبل السريرية والدراسات داخل الكائن الحي *(in vivo)* للتحقق من فعاليته وسلامته.

كلمات مفتاحية: سرطان القولون والمستقيم، شجرة دم الأخوين، دورة الخلية، الموت المبرمج (الاستماتة)، الالتهام الذاتي.

قسم العلوم الفسيولوجية. كلية الطب والعلوم الصحية. جامعة عدن

Introduction

Colorectal cancer remains a significant global health challenge, with high incidence and mortality rates despite advances in conventional therapies. Standard treatments such as chemotherapy and radiotherapy often cause severe side effects and may encounter drug resistance, highlighting the need for safer, more effective alternatives [1]. The most commonly used drugs in Colorecta Cancer (CRC) treatment include 5-fluorouracil (5-FU), capecitabine (Xeloda), irinotecan (Camptosar), oxaliplatin (Eloxatin), and trifluridine/tipiracil (Lonsurf), which primarily act by interfering with DNA synthesis and arresting the cell cycle, particularly during the S-phase [2]. However, these agents are often associated with severe side-effects such as gastrointestinal toxicity, myelosuppression, and peripheral neuropathy, which negatively impact patients' quality of life and treatment compliancev [3]. In response to these limitations, increasing attention has been directed toward natural products that possess anticancer potential with reduced toxicity.

Dracaena cinnabari balf. f. (DC), endemic to Socotra Island in Yemen, produces a red resin known as Dragon's Blood. This resin has been used in traditional medicine for centuries across various cultures - including Greek, Roman, Arab, and Latin American - for treating wounds, ulcers, gastrointestinal disturbances (e.g., dysentery, diarrhea), infections, and inflammatory conditions. These applications are supported by ethnopharmacological knowledge and preliminary biomedical findings [4]. Phytochemical investigations of *DC* resin have revealed various bioactive constituents including flavonoids, biflavonoids (e.g., cinnabarone), triflavonoids (e.g., damalachawin), sterols, and triterpenoids [5,6]. These compounds are known for their antioxidant, antimicrobial, and anti-inflammatory activities, and have demonstrated potential cytotoxicity against cancer cells in preliminary studies [7-10].

Although the DPPH assay does not directly assess anticancer activity, it provides mechanistic insight into the extract's redox-modulating potential. Given the critical role of oxidative stress and redox signaling in cancer cell survival, apoptosis, and autophagy, the observed antioxidant activity supports the biological plausibility of the extract's cytotoxic effects, rather than serving as a direct indicator of anticancer efficacy [11].

Apoptosis, a regulated and non-inflammatory form of programmed cell death, plays a crucial role in eliminating malignant cells during chemotherapy and is widely recognized as a fundamental mechanism by which many anticancer agents exert their cytotoxic effects [12]. In colorectal cancer, chemotherapeutic drugs such as 5-FU, oxaliplatin, and irinotecan induce apoptosis by interfering with DNA synthesis and disrupting key regulatory proteins, thereby promoting cancer cell death and enhancing treatment efficacy [2]. The therapeutic success of these agents depends not only on their capacity to arrest cell proliferation but also on their ability to selectively activate apoptotic pathways in cancerous cells. In addition to apoptosis, autophagy has emerged as a critical regulator in cancer biology.

Autophagy is a highly conserved lysosomal degradation pathway that maintains cellular homeostasis by removing damaged organelles and proteins [13]. While autophagy can promote tumor cell survival under metabolic stress, it may also contribute to cell death depending on the cellular context [1,14,15]. This duality has positioned autophagy as a potential therapeutic target, particularly in drug-resistant cancers. In this study, methanolic extract of DC is investigated for its anti-proliferative activity as well as its induction of apoptosis and autophagy against human colorectal cancer (HT-29) cells. Research findings may lead to explore the mechanistic role of DC in comparison to the conventional chemotherapeutic 5-FU, including assessment of their combined efficacy in inducing apoptosis. Understanding how DC modulates key cellular processes such as cell cycle regulation, apoptosis, and autophagy may provide valuable insights into its potential as a complementary anticancer agent.

While existing studies have largely focused on the cytotoxic effects of Dragon's blood in cancer models other than colorectal cancer, this study was designed to address the research gaps by systematically evaluating the effects of DC on colorectal cancer cells and comparing its mechanisms of action with those of the standard chemotherapeutic agent 5-fluorouracil using flow cytometric analysis. It provides a strong scientific basis for assessing the therapeutic potential of Dragon's blood prior to the interpretation of experimental outcomes.

Methods

EXTRACT PREPARATION

Resin from DC was collected from its natural habitat on Socotra Island, Yemen, during June–August 2023. The extract was prepared using a standardized extraction protocol under controlled conditions. First, the resin was manually ground and then pulverized into a fine powder using an electric grinder. Then, the DC powder (approximately 250 g) was macerated in 1.25 L of methanol at room temperature overnight and then filtered. The methanolic extract was collected and evaporated under reduced pressure at 40°C. This extraction process was repeated twice, yielding a total of 224.51 g of sticky red extract. The crude extract was dried using a rotary evaporator and stored at −20°C until further use [16,17]. The extract was used as a whole to reflect its traditional application and to evaluate the combined biological effects of its bioactive constituents**.**

Data Analysis

Data were obtained from triplicate technical replicates and expressed as mean±SD of three technical replicates (n=3). Statistical evaluation was performed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons against the control group. Statistical significance was set at $p < 0.05$. It should be noted that the replicates represent repeated measurements within the same experimental run; therefore, the analysis assesses technical reproducibility rather than inter-experimental biological variation.

Results

DRAGON'S BLOOD RESIN METHANOL EXTRACT (DC) INHIBITS THE GROWTH OF HUMAN COLORECTAL CANCER CELLS (HT-29)

The anti-proliferative effects of DC and 5-FU on HT-29 cells were evaluated using the *Sulforhodamine b* (SRB) assay after 72 hours of exposure to different concentrations as shown in Table 1. Both DC and 5-FU inhibited cell growth in a dose-dependent manner. DC exhibited minimal cytotoxicity at low concentrations, but cytotoxicity increased sharply at higher concentrations, resulting in a significant decline in cell viability with an IC_{50} value of 31.82 µg/mL (Figure 1a, Table 1a). In comparison, 5-FU showed a gradual decrease in viability with increasing concentration, reaching an IC_{50} value of 14.61 µg/mL (Figure 1(b) and Table 1(b).

Table 1: Assessment of the anticancer activity by the SRB assay of HT-29 cell viability when using (a) DC extracts and (b) 5-FU

	Raw data			Blank Corrected Data			Viability %			Mean	ST	Significance vs control
DC					(A)							
Concentration µg/mL	**1**	**2**	**3**	1	2	3	1	2	3			
Control	3.14	3.15	3.13	3.11	3.11	3.09	100	100	100	100	**0**	-
0.01	3.18	3.19	3.17	3.15	3.16	3.14	101.36	101.76	100.98	101.37	**0.32**	**ns**
0.1	3.16	3.17	3.16	3.13	3.14	3.13	100.64	101.02	100.64	100.77	**0.18**	**ns**
1	3.13	3.14	3.14	3.09	3.10	3.10	99.59	99.94	99.94	99.82	**0.16**	**ns**
10	3.10	3.11	3.11	3.07	3.08	3.08	98.77	99.09	99.09	98.99	**0.15**	**ns**
100	0.07	0.06	0.07	0.04	0.02	0.04	1.28	0.79	1.23	1.098	**0.22**	*******
Blank	0.03	0.03	0.03	**Blank Average**		0.03	**Control average**		3.11			

5-FU					(B)							
Concentration µg/mL	**1**	**2**	**3**	1	2	3	1	2	3			
Control	3.11	3.16	3.11	3.08	3.12	3.08	100	100	100	100	**0**	-
0.01	3.16	3.18	3.14	3.12	3.15	3.10	100.95	101.71	100.23	100.96	**0.60**	**ns**
0.1	3.11	3.08	3.05	3.07	3.04	3.01	99.25	98.30	97.42	98.32	**0.75**	**ns**
1	2.70	2.51	2.65	2.67	2.47	2.61	86.20	79.88	84.52	83.54	**2.67**	*******
10	2.16	1.92	2.02	2.13	1.88	1.98	68.72	60.93	64.16	64.60	**3.20**	*******
100	0.24	0.19	0.29	0.20	0.15	0.26	6.52	4.96	8.36	6.61	**1.39**	*******
Blank	0.03	0.03	0.04	**Blank Average**		0.04	**Control average**		3.09			

ns: not significant ($p > 0.05$); ***$p < 0.001$ versus control.

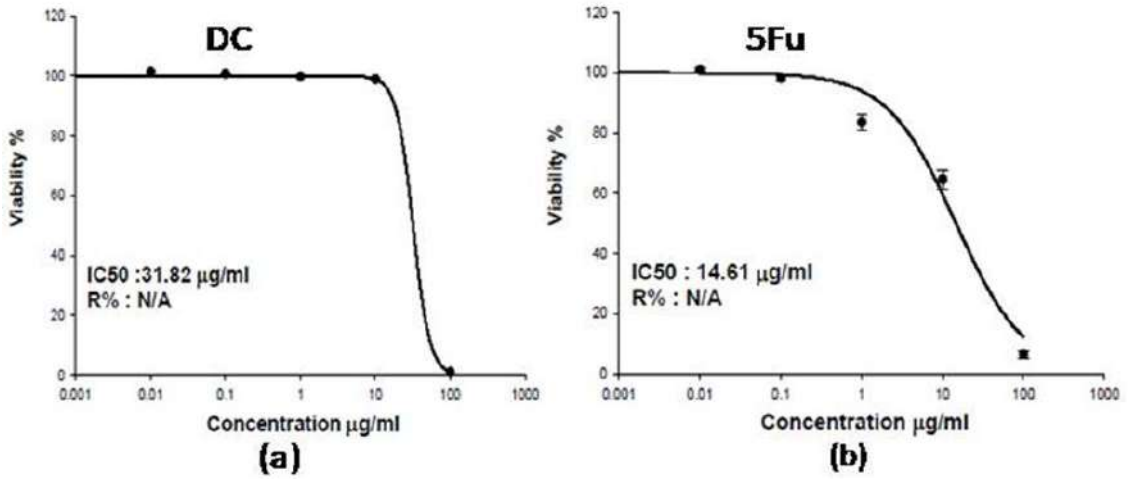
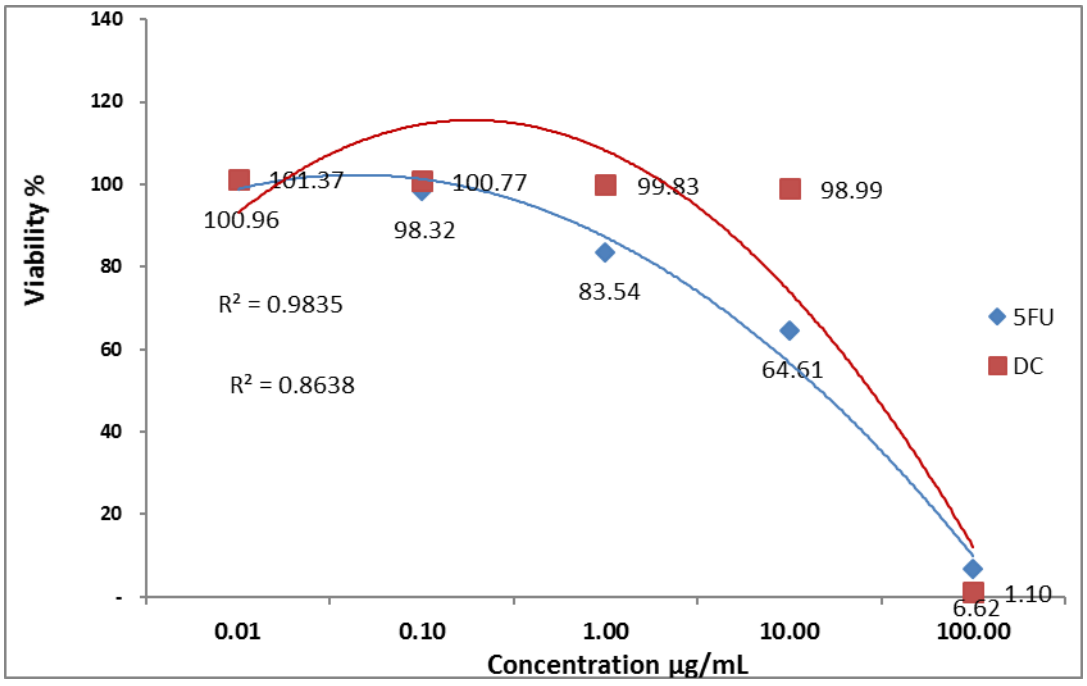
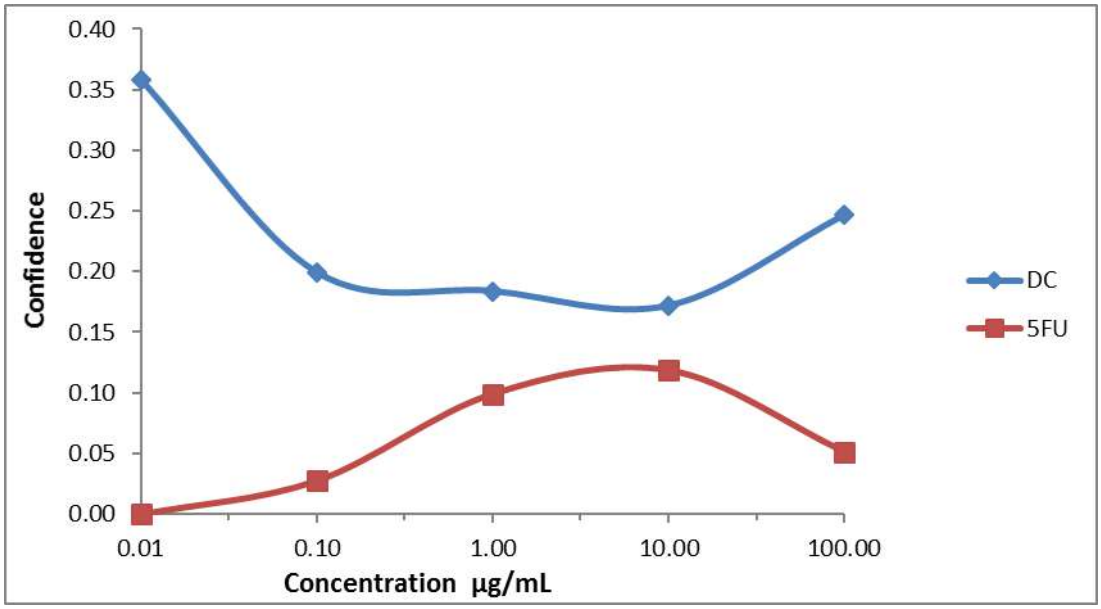


Figure 1: Plot of Percentage Viability of HT-29 Cells Against the Concentration (µg/ml) of (a) DC Extract (b) 5-FU. The IC_{50} Values Included

The findings indicate that DC contains bioactive compounds capable of inducing cytotoxicity in colorectal cancer cells, with a confidence value indicated in Figure 2(ii).



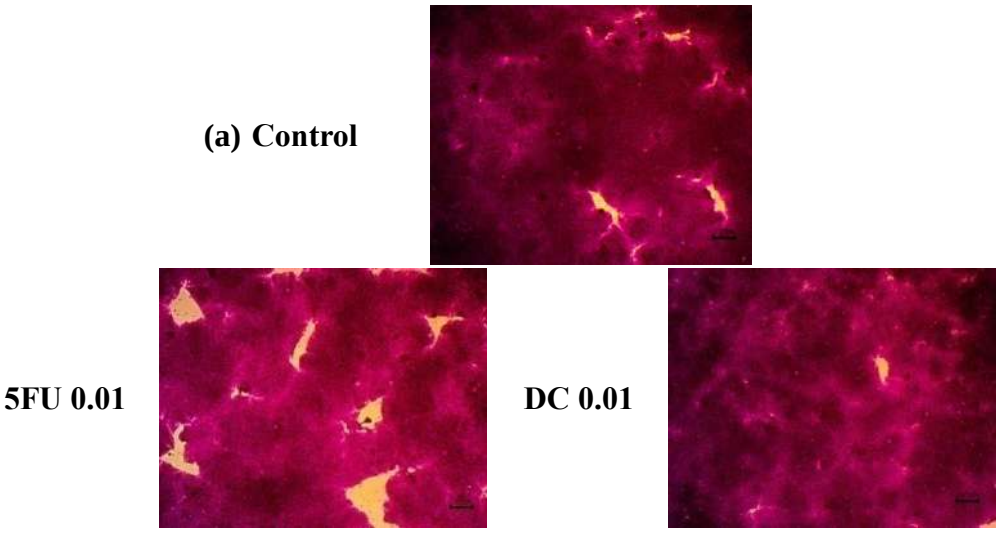
(i)



(ii)

Figure 2: (i) Dose-dependent inhibitory effects of DC and 5-FU on HT-29 cell viability.
(a) Cell viability percentage in response to varying concentrations of DC. **(b)** Cell viability percentage in response to 5-fluorouracil (5-FU).
(ii) Confidence Interval: (DC) show a high level of confidence for its IC50.
IC_{50} values are indicated.

The results suggest a need for further purification and characterization to enhance its therapeutic efficacy. Optical microscope stained images of cytotoxicity assays for anticancer activity of HT-29 cell lines as shown in Figure 3.



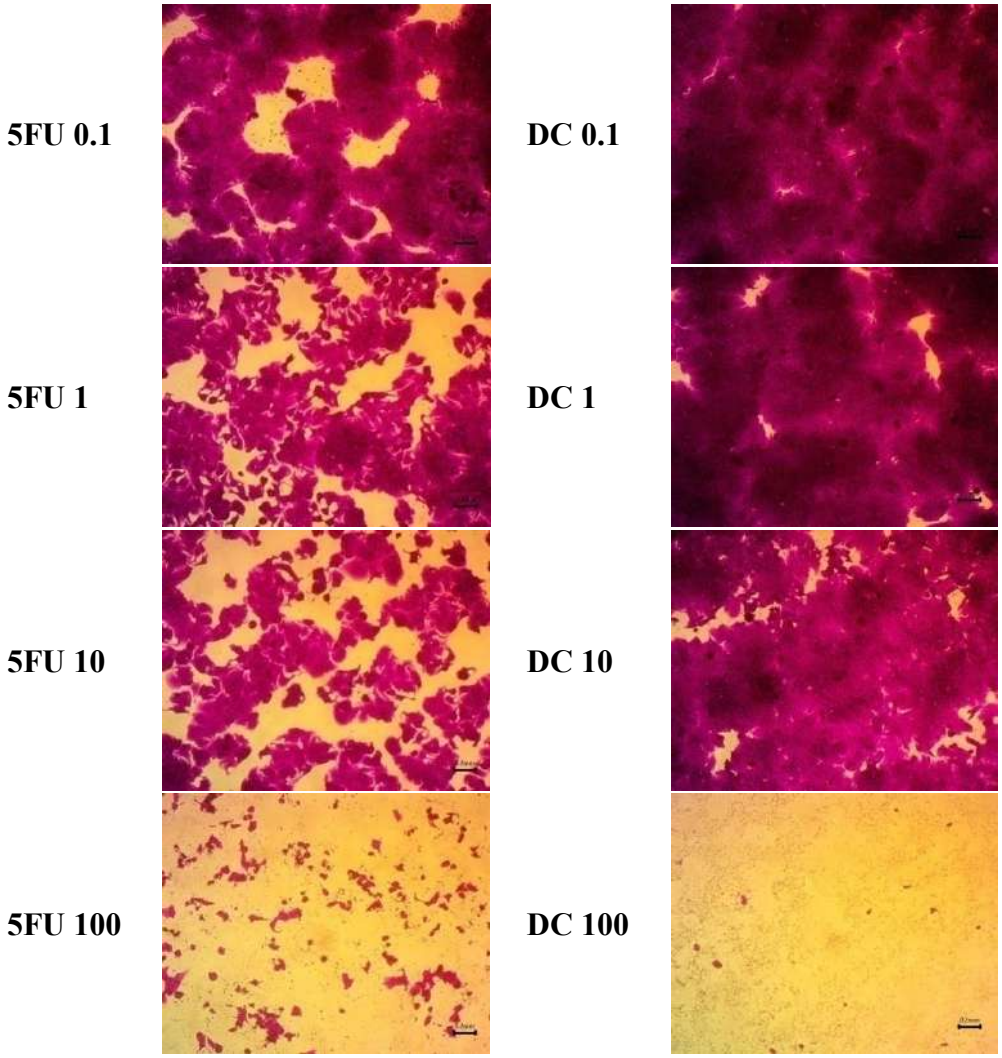


Figure 3: Anticancer activity: The optical microscope stained images of cytotoxicity assays of HT-29 cell lines: (a) control (b) 5-Fu (c) DC.

INDUCTION OF APOPTOSIS BY DRAGON'S BLOOD RESIN TREE

Figure 4 shows control cells, cells treated with different concentrations of 5-FU, and cells treated with different concentrations of DC.

Apoptosis was evaluated in three replicates of HT-29 cells using Annexin V-FITC/PI dual staining and flowcytometry.

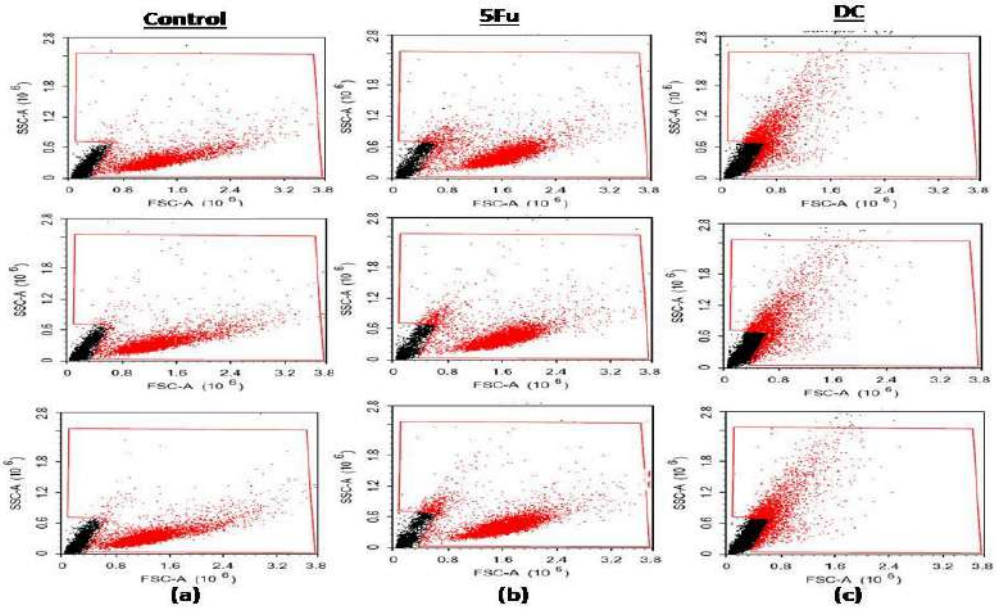


Figure 4: Apoptosis: A two-parameter profile of side scatter (SSC) vs. forward scatter (FSC), using the pulse area (A) is used on three replicas of HT-29 cell line for (a) Control (b) 5-FU (c) DC.

DC significantly increased the percentage of apoptotic cells compared to untreated cells and cells treated with 5-FU. Late apoptosis was observed in an average of 83.21% of DC-treated cells, whereas 5-FU induced late apoptosis in 15.57% of cells. Necrosis also increased significantly from 2.93% in control cells to 16.68% in DC-treated cells as illustrated in Figure 5.

Therefore, the DC-induced late apoptosis and necrosis were approximately 5.3-fold higher than 5-FU and 54.18-fold higher than the control. In contrast, 5-FU induced early apoptotic cells in 7% of the total population, whereas DC did not significantly induce early apoptosis (Figure 5).

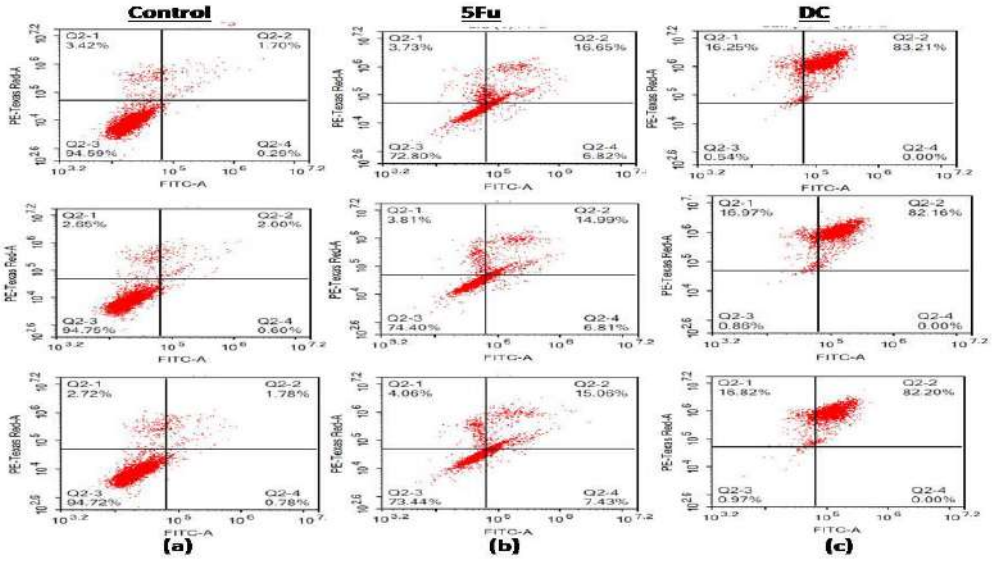


Figure 5: Apoptosis: Flowcytometric detection of apoptosis in three replicas of HT-29 cell line. Cells treated with the respective drug regimens and untreated controls were dual-labeled with propidium iodide (PI) and Annexin V fluorescence and

analyzed by flow cytometry. (a) Untreated Control cells (b) Cells treated with 5-fluorouracil (5-FU) (c) Cells treated with Dragon's Blood Methanolic extract (DC). Lower left quadrants: live cells; lower right quadrants: early apoptotic cells; upper left quadrants: necrotic cells; upper right quadrants: late apoptotic cells. The percentage of apoptotic cells is indicated on the plots.

INDUCTION OF AUTOPHAGY BY DRAGON'S BLOOD RESIN

Autophagy was assessed using acridine orange staining of acidic vesicular organelles, followed by flow cytometry (λ Excitation/Emission = 488/530 nm) in three replicates of HT-29 cells. Control cells showed minimal fluorescence intensity, establishing the baseline autophagy level. Treatment with 5-FU increased fluorescence intensity by 1.5-fold compared to control cells, indicating moderate autophagy activation. DC treatment, however, resulted in approximately twice the fluorescence intensity of 5-FU, demonstrating a stronger induction of autophagy (Figure 6,7). These findings suggest that DC enhances cancer cell death through both apoptotic and autophagic pathways.

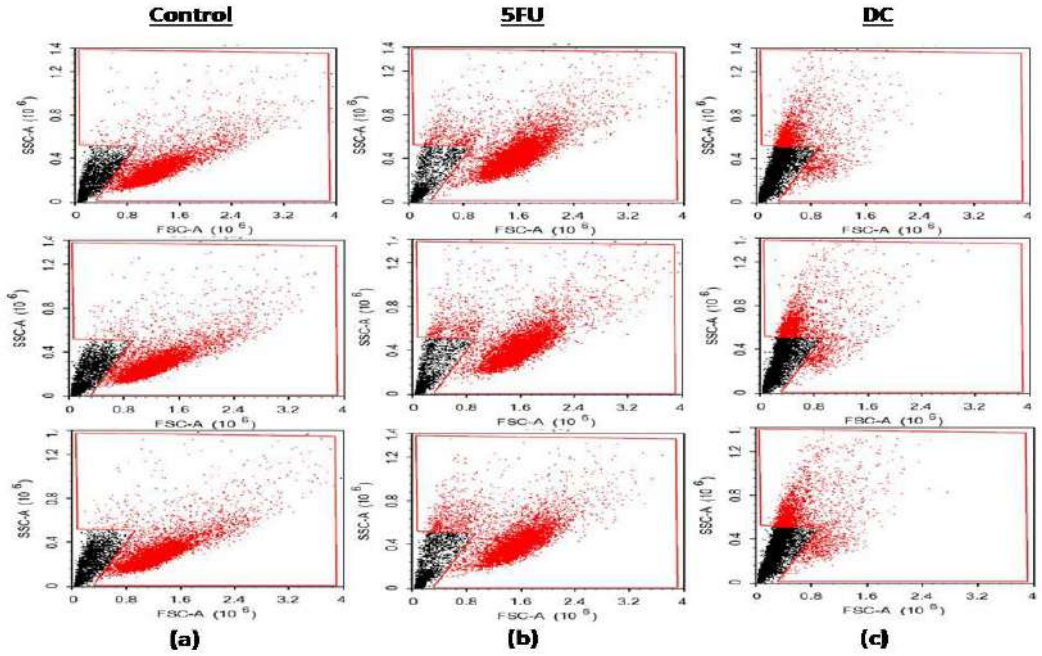


Figure 6: Autophagy: The FSC-A/SSC-A diagram is a dual parameter contour plot of side scatter (SSC) vs. forward scatter (FSC), using the pulse area (A) is used on three replicas of HT-29 cell line (n=3) for (a) Control (b) 5-FU (c) DC.

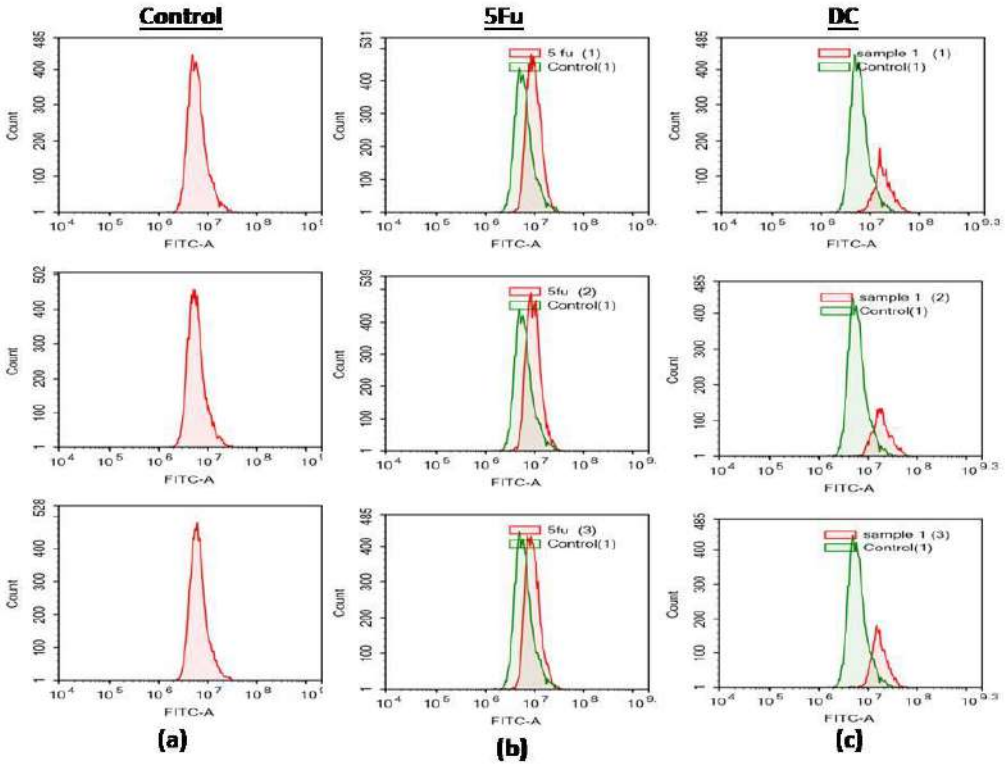


Figure 7: Autophagy: Flow cytometry histogram of cell count versus FITC intensity (FITC-A denotes FITC area) of (a) Untreated Control (b) FITC-labeled 5-fu added to HT-29 cells. (c) FITC-labeled DC added to HT-29 cells.

3.4. EFFECTS OF DC ON CELL CYCLE DISTRIBUTION

The effect of DC on cell cycle progression was analyzed in three replicates of HT-29 cells (Figures 8 and 9).

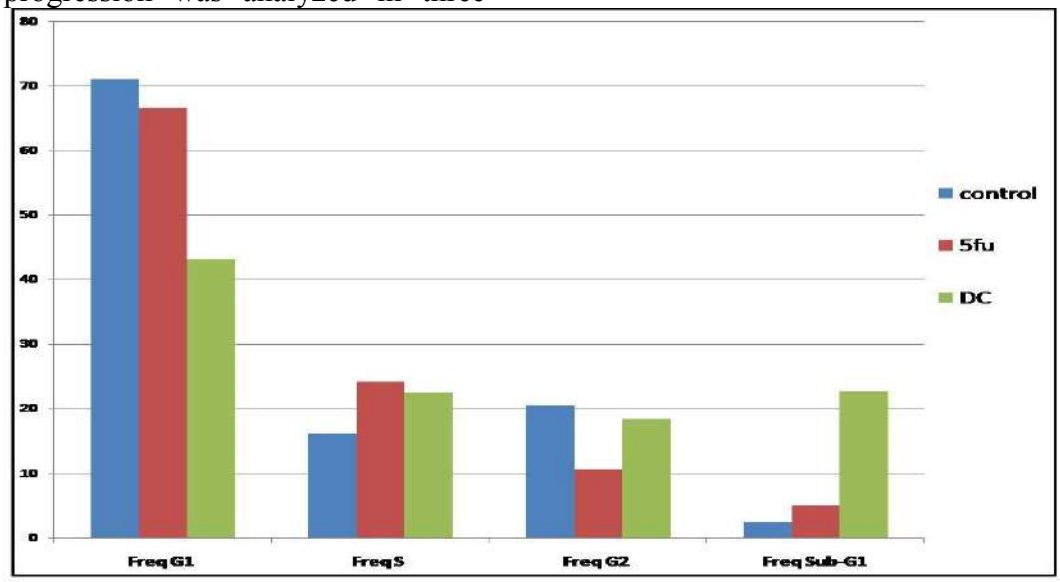


Figure 8: The proportion of each phase in a cell cycle distribution of HT-29 for control cells, 5-FU-treated cell, and DC-treated cells (n=3).

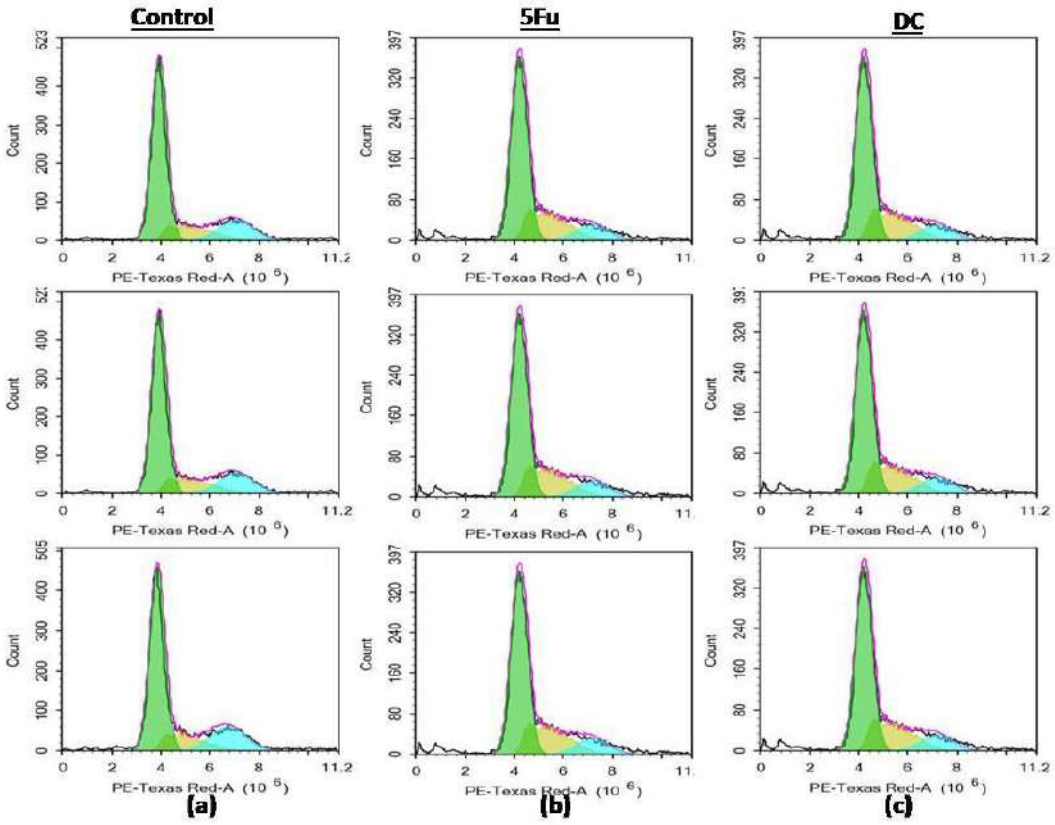


Figure 9: Histograms of cell cycle of HT-29 cell line.

Both DC and 5-FU induced cell cycle arrest in the S-phase compared to control cells. DC increased the percentage of cells in S-phase from 16.1 ± 1.6% to 22.4 ± 1.8%, while 5-FU increased it to 24.2 ± 1.0%. S-phase arrest was associated with a decreased cell population in G2/M, which dropped from 20.4 ± 1.3% in controls to 18.3 ± 1.2% for DC and 10.6 ± 1% for 5-FU. Additionally, DC significantly increased the dead cell population in the Sub-G1 phase from 2.5 ± 0.1% to 22.7 ± 0.6%, whereas 5-FU treatment resulted in only 5.0 ± 0.6% in Sub-G1. Overall, DC treatment led to an accumulation of cells in the S and Sub-G1 phases with a reduction in G1 phase cells. The increase in the Sub-G1 population likely reflects DNA fragmentation in apoptotic cells, confirming apoptosis induction by DC [25].

3.5. ANTIOXIDANT ACTIVITY OF DRAGON'S BLOOD RESIN

The antioxidant potential of Dragon's Blood resin was evaluated using the DPPH assay. The resin demonstrated dose-dependent radical scavenging activity at concentrations of 15.625–250 µg/mL, with Trolox (3.906–31.25 µg/mL) used as a positive control (Figure 10, Table 2). After 30 min incubation at room temperature in the dark, the reduction in DPPH absorbance at 540 nm was measured. The percentage of inhibition was calculated using the standard formula, and IC_{50} values were determined via nonlinear regression in GraphPad Prism 9 (log[inhibitor] vs. normalized response, variable slope) (Figure 10). The results indicate that Dragon's Blood resin possesses moderate free radical scavenging activity, confirming its potential as an effective natural antioxidant.

Table 2: DPPH Inhibitory Concentration (a) Standard Trolox and (b) DC Extracts

(A) Standard Trolox					
Conc (µg/mL)	3.90625	7.8125	15.625	25	31.25
	8.07	21.09	51.27	79.57	85.17
%inhibition	8.4	20.76	53.54	79.24	85.99
	9.39	23.56	50.74	77.92	84.51
(B) Dragon's Blood Resin					
Conc (µg/mL)	15.625	31.25	62.5	125	250
	9.44	22.82	43.2	68.49	81.07
%inhibition	9.71	23.21	44.26	67.3	80.67
	10.37	21.09	42.14	58.69	80.94

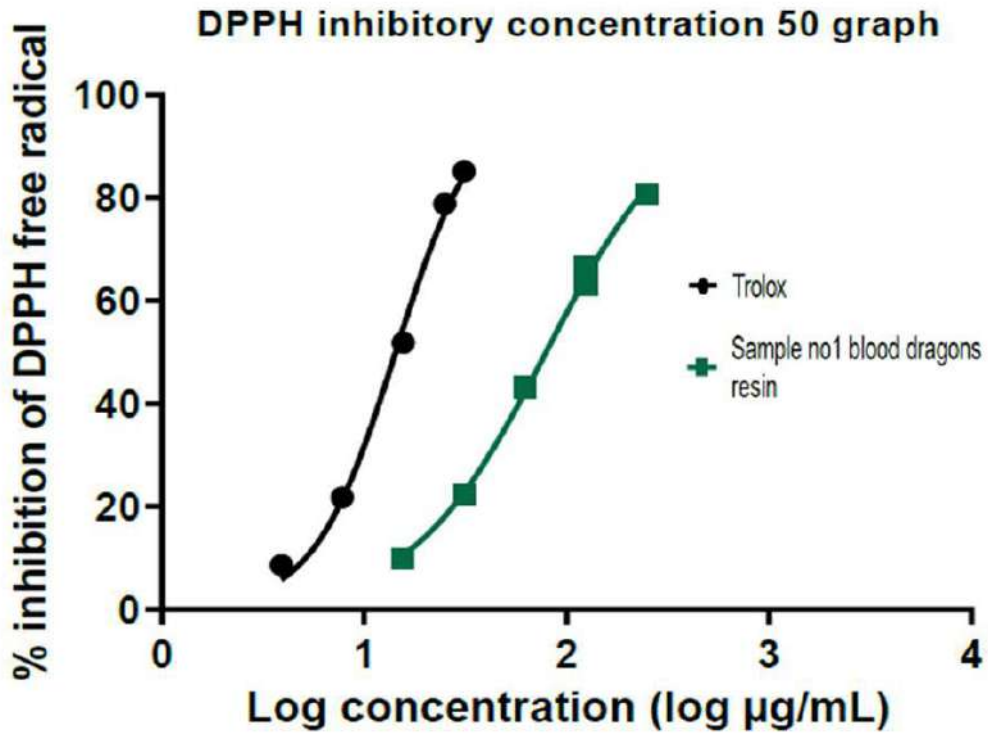


Figure 10: DPPH Radical Scavenging Activity of Trolox with IC50 = 14.31 and DC Extracts with IC50 = 79.22 (n = 3). (STD Error =1.02)

Discussion

Numerous studies have demonstrated that anti-proliferative effects of cytotoxic agents are often linked to their ability to induce cell cycle arrest at specific phases, ultimately leading to apoptosis [26-28]. In other words, cell cycle arrest is a critical mechanism employed by many antitumor drugs, including 5-FU, cisplatin, and doxorubicin [29]. In this study, SRB assay results assert the vigorous cytotoxic ability of DC

against HT-29. DC seems to engage effectively with cellular targets. At high concentrations, it can induce significant cytotoxicity by targeting specific cancer cell mechanisms. The higher value of DC's IC50 compared to 5-FU's IC50 may be due impurity and ineffective substances in the extract.

In Figure 8, the low cell rate of DC in G1 when compared to untreated cells and 5-FU treated cells could be explained by the high rate of autophagy and programmed death (apoptosis). 5-FU is known for its effectiveness in inducing S-phase arrest. For 5-FU, the level of treated cancer cells is higher than that of untreated cancer cells in S-phase.[1,15] It indicates that slowing down or stopping the progression of the cell cycle may be due to DNA damage during its replication in the S stage. DC seems to have the same effects as it has almost the same level in S-phase as 5-FU. which indicates that it may have the same effects in S-phase as 5-FU. Thus, DC effectively induced S-phase arrest. In sub G1, a key indicator of apoptotic cell death, the high level of cells treated with DC indicates killing a markedly large number of cells. It may indicate that DC interferes with DNA synthesis, halting cell cycle progression and leading to apoptosis. In G2/M, the cell rate of 5-FU is low due to S-phase checkpoint. The rate for DC is close to the untreated cells in G2/M which could be explained as cells could not pass G2/M checkpoint which explains the high level of Sub-G1 (i.e. cells arrested in G2 do not complete their way to mitosis till they die). Thus, DC effectively induced S-phase arrest and markedly increased the sub-G1 cell population. The low effects of 5-FU on apoptosis can be due to disruptions in TP53 or BAX in a colon cancer cell line which let cells become resistant to apoptosis [1-29].

On the other hand, flowcytometric detection of late apoptosis and necrosis in this study shows a great capacity of DC in inducing apoptosis and necrosis compared to 5-FU. To our knowledge, there is no study that has specifically examined the autophagic response of HT-29 cells to DC treatment.

Autophagy is often described as a double-edged sword that can promote cell survival and cell death depending upon the context studied [13,30,31]. One of its fundamental function in eukaryotic life is as a survival mechanism that allows cells to cope with harsh conditions, stress, or hypoxia by degrading damaged organelles or proteins to provide essential amino acids [32-35]. Autophagy also induces cell death. The level of autophagic flux in cells plays a role in both a cell survival and cell death [36,37]. Basal autophagic flux will promote cell survival whereas elevated and prolonged autophagic flux will promote cell death [39]. In this study, DC demonstrated superior autophagic results which potentially contribute to cancer cell death as indicated by the high rate of apoptosis [11]. The observed increase in fluorescence intensity following DC treatment suggests greater disruption of cellular homeostasis (Figures 6 and 7) and high levels of apoptosis (Figure 5) and sub-G1 value (Figure 8). Further investigation may be needed determine whether autophagy synergizes with apoptosis or counteracts it.

In addition to its anticancer effects, DC also demonstrated moderate antioxidant activity in the DPPH antioxidant assay (Figure 10).

Antioxidants play a critical role in mitigating oxidative stress by free radical Reactive Oxygen Species (ROS), which are major contributors to DNA damage. This reduction in oxidative damage prevents mutations that could initiate cancer development. Additionally, antioxidants help maintain cell cycle integrity by shielding DNA and regulatory proteins (e.g., p53 and Rb) from oxidative damage [40]. Furthermore, antioxidants contribute to cancer prevention by enhancing apoptosis [2,42,43]. Tested DC in this study had a moderate activity (IC50=79.22 µg/mL). Although the antioxidant effect alone does not explain the cytotoxicity observed, it may contribute to the overall multitarget profile of DC. These findings highlight DC as a multi modal anticancer agent capable of inducing S phase arrest, apoptosis, and autophagy in colorectal cancer cells. By integrating cell cycle disruption with programmed cell death pathways, DC demonstrates potential as a complementary or adjuvant therapeutic candidate alongside conventional chemotherapy.

Conclusion

The coordinated induction of cell cycle arrest, apoptosis, and autophagy observed in this study indicates that DC extract exerts its anticancer effects through a multi-target cellular mode of action rather than a single molecular pathway. This integrated modulation of multiple, interconnected cellular processes is a characteristic feature of phenolic-rich plant extracts and may account for their enhanced anticancer efficacy by simultaneously disrupting redox homeostasis, mitochondrial function, and programmed cell death signaling, thereby potentially limiting the development of chemo-resistance.

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