BIOSCIENCE JOURNAL

SYNERGISTIC EFFECT OF SORAFENIB WITH *Platycladus orientalis* (L) LEAF EXTRACT ON CERVICAL CANCER

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How to cite: SAMUEL, E., et al. Synergistic effect of Sorafenib with *Platycladus orientalis* (L) leaf extract on cervical cancer. *Bioscience Journal*. 2023, **39**, e39011. https://doi.org/10.14393/BJ-v39n0a2023-62558

Abstract

The extracts of Platycladus orientalis (L.) Franco leaves have shown promising anti-cancer, anti-oxidant and anti-inflammatory potency with the traditional knowledge of healing HPV associated warts. The purpose of this research is to assess the synergistic activity of sorafenib and Platycladus orientalis (L) leaf extraction on cervical cancer cells. The cytotoxicity efficiency of different concentrations of Sorafenib and ethanol extract of Platycladus orientalis (L.) leaves were tested on HeLa cells by MTT and Trypan blue assays. The synergistic effect of the IC_{50} concentrations of Sorafenib and Platycladus orientalis (L.) on HeLa cell by MTT assay, and mRNA expression levels of tumor suppressor tazarotene-induced gene 3 (TIG3), proliferating cell nuclear antigen (PCNA) gene and apoptosis modulator (Bcl-2) gene by RT-PCR were evaluated with individual treatments. Combination treatment showed a relatively more expression of TIG3 and less expression of Bcl-2 and PCNA was observed. Growth factor-induced MAPKP activation was arrested by compound combination treatment, which and suppression of proliferation-induced apoptosis of cervical cancer cells. Based on the our results, the combination of sorafenib and crude leaf extract from Platycladus orientalis (L.) can effectively suppress cervical cancer cell growth, thereby providing an interesting rationale for further clinical trials and *in-vivo* studies.

Keywords: Bcl-2, Franco. HeLa cells. PCNA. Platycladus orientalis (L.). Sorafenib. TIG3.

1. Introduction

A malignant tumour that affects the lower portion of the uterus is called cervical cancer, and HPV infection is its main cause. A compromised immune system, smoking, and use of miscarriage prevention medications are further risk factors (Bermudez et al. 2015). Cervical carcinoma is common type of cancer with around 500 thousand new cases diagnosed each year across developing and underdeveloped nations in women (Bray et al. 2018). Women between the ages of 35 and 44 are more prone to be diagnosed with cervical cancer. Approximately 20% of instances are identified in women over 65, and it seldom occurs in women under the age of 20 (Howlader et al. 2017). The commonly used chemotherapy and radiation therapy are often associated with serious side effects of morbidity. It has been highlighted that the rate of recurrence of invasive cervical cancer which is caused majorly by infection with high-risk type HPV 16/18 is high and the recurrence needs to be prevented by effective treatment (Ghaemmaghami et al. 2012).

Hence novel targeted treatment and prevention approaches are urgently needed for early-stage and post-therapy treatments of cervical cancer (Manci et al. 2011; Huang et al. 2012; Tewari et al. 2017).

Sorafenib is an oral multi-kinase inhibitor, categorized as a molecularly targeted drug (Wilhelm et al. 2004). It is used for the treatment of renal cell carcinoma, hepatocellular carcinoma, and thyroid carcinoma. Sorafenib is FDA-approved for the treatment of cancer but it is known to cause certain severe side effects: diarrhea, fatigue, hand-foot skin reaction, hypertension, and adverse effects such as cardiac failure and coronary artery disease that could even lead to death (Llovet et al. 2008). Given the fact that Sorafenib is a synthetic compound that possesses many side effects, the interest of the researchers has switched towards combinational therapies involving natural herbal medicine to treat cancer which may quench the toxic reactions and poses little to no side effects. Furthermore, the complementation of such molecular therapeutic drugs with plant extracts that have anti-cancer potency will result in increasing the efficiency of synthetic drug action. To ascertain the cytotoxic impact and molecular activity of various plant extracts, much study has been conducted. Inflammation is one of the most important factors that contributes to the progression of cancer, and Platycladus orientalis (L.) , a monotypic genus of evergreen coniferous tree in the Cupressaceae family, was discovered to reduce inflammation by stopping NF-B activation (Basyal et al. 2018; Kim et al. 2013). The research studies on the extract obtained from Platycladus orientalis (L.) Franco has shown it to be a promising compound that has great potential against various health problems such as cough, bronchitis, asthma, skin infection, excessive menstruation, and premature baldness. Research has also emphasized its anti-oxidant, anti-cancer, and anti-inflammatory properties (Gan et al. 2021; Srisaikham 2021). It has also exhibited anti-cancer properties on breast cancer and leukemia cells (Amirghofran and Karimi 2001; Guleria et al. 2008; Zhang et al. 2013). This plant's essential oil has been studied for its ability to suppress the Severe Acute Respiratory Syndrome (SARS) (Loizzo et al. 2008; Srivastava et al. 2012). The mitogen activated protein kinase (MAPK) pathway is associated with wide range of human malignancies (Beeram et al. 2005), which ultimately, modulates the activity of transcription factors (Kolch 2000; Chang et al. 2003) and further leading to higher cell proliferation, cell cycle progression and inhibition through apoptosis (Huether et al. 2006). Recently, it has been proven that targeting the MAPK pathway has a wide range of activity on apoptosis, differentiation, and cell proliferative signals and can be targeted for tumor development and progression therapy (Kohno and Pouyssegur 2003; Rubinfeld and Seger 2005; English and Cobb 2002). Sorafenib is known to inhibit the components of the MAPK pathway such as RAS/RAF/MEK/ERK signaling pathway that controls proliferation besides, VEGFR-2 signaling pathway that blocks tumor angiogenesis (Liu et al. 2006). The key marker of human cell proliferation is the PCNA and the key marker of inhibition of apoptosis is an elevated Bcl-2 protein (Cardano et al. 2020; Bierbrauer et al. 2020). The tumor suppressor, TIG3 is suppressed or negatively regulated by the activated ERK/MEK pathway in cancer cells and the re-expression of TIG3 mRNA caused inhibition of ovarian cancer cells (Lotz et al. 2005). In normal keratinocytes, the TIG3 mediates the terminal differentiation as well as apoptosis and its loss leads to cancerous transformation and proliferation (Scharadin and Eckert 2014). Current study was hence focused on evaluating the synergistic effect of sorafenib drug with ethanol extract of Platycladus orientalis (L.) on the growth of HeLa cancer cell line, as well as the molecular action on regulating the mRNA levels of TIG3, PCNA, and Bcl-2.

2. Material and Methods

Cell line and Reagents

The HeLa cells were obtained from the National Centre for Cell Science, Pune, India. Dulbecco's Modified Eagle Medium (DMEM), Trypan blue, Dimethyl sulfoxide (DMSO), Fetal Bovine Serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and trypsin-Ethylenediamine tetraacetic acid were purchased from Hi media, India. Pen-Strep antibiotics were obtained from Thermo Scientifics, MA USA. Sorafenib Tablets, Taq DNA Polymerase 2xmaster mix, iScript™ cDNA synthesis kit (Bio Rad) and TRIzol reagent were procured from Netmeds, Ampliqon and Life Technologies, respectively.

Plant Collection and Authentication

The Botanical Survey of India, Coimbatore authenticated (BSI/SRC/5/23/2020/Tech./568) the samples as Platycladus orientalis (L.) Franco - CUPRESSACEAE collected from the Karunya Institute of Technology and Sciences campus.

Phytochemicals Extraction from Platycladus orientalis (L.) Franco

The Leaves of Platycladus orientalis (L.) Franco was maintained in an incubator at 55°C for 24hr and crushed with a grinder. further, to attain a vast range of compounds, 25 grams of pulverized leaves were used for extraction with 250 ml of 95% of ethanol, at 55°C for about 17 cycles with soxhlet apparatus and further segreated by using a rotatory vacuum evaporator (Singh and Singh 2009). The dried extract was weighed and stored at −20 °C. The concentration of 2.4, 1.2, 0.6, 0.3 and 0.15 mg/ml were prepared using 100 % dimethyl sulfoxide (DMSO) and the cancer cell growth analyses were carried out by referring to the relevant literature (Amirghofran and Karimi 2001).

Preparation of Sorafenib

Stock solutions of Sorafenib were made by using DMSO and kepted at −20 °C. Further dilution was prepared using DMSO to make the following concentration of 1-10 µM (Huether et al. 2007; Lin et al. 2016).

Cell culture

HeLa cells were cultured in complete DMEM media (10% FBS and 1% pen-strep) and kept in a 5% CO_2 incubator at 37°C (Pirouzfar et al. 2020).

In-vitro Tumor cell growth inhibition studies

Cytotoxicity assay (MTT assay)

The MTT assay was carried out to assess the effect of Sorafenib and Platycladus extract on HeLa cells. 96-well plates seeded with 1.2×10^4 cells/well were subjected to CO_2 incubation overnight. Further, the cells were subjected to treatment with different concentration i.e (1-10 µM) Sorafenib and (2.4, 1.2, 0.6, 0.3, 0.15 mg/mL) Platycladus orientalis (L.) for a period of 24h. The cells were subjected to PBS wash and 40 µl of MTT (0.5 mg/mL) were added to each well and kept for 4h. Further, 100 µL DMSO was added before absorbance was recorded at 570 nm (Chen et al. 2006) and the cell viability percentage was calculated using the following formula,

$$\text{Cell viability \%} = \left[\frac{\text{Test sample OD}}{\text{Control OD}}\right] \text{X } 100$$

Trypan blue exclusion assay

Exclusion assay was performed to analyze the cell viability by using 1×10^5 cells/well (6-well plate) and incubated overnight in CO_2 incubator. Further, treatment was given for 24h & 48h with various concentrations of Sorafenib and Platycladus orientalis (L.) extract both individually and in combination. Later, the cells were centrifuged at 4000 xg for a duration of 5 min and the pellets were subjected to PBS wash and staining with trypan blue for cell counting with a hemocytometer (Sanfelice et al. 2017).

Drug combination studies

To examine the synergistic effects on growth inhibition of HeLa cells, the combination treatment of sorafenib (IC_{50} - 5 µM) with Platycladus orientalis L. (IC_{50} - 0.6 mg/ml) in the equal ratio was studied by MTT and Trypan blue assays as per the experimental design by following the protocol that is mentioned above. Further, to study the synergistic effects on regulating the mRNA levels of TIG3, PCNA, and Bcl-2, the same experimental along with the medium were placed in incubator for a duration of 24h in presences of CO_2 (Lin et al. 2016; Chen et al. 2006). Then the cells were subjected to RNA isolation and cDNA synthesis by reverse transcriptase PCR.

The study was carried out as per the experimental groups given as mentioned in Table 1.

Table 1. Experimental Groups and Treatments.

Group	Treatment
I	Untreated HeLa Cells
II	Sorafenib + *Platycladus orientalis L*. Treated HeLa Cells
III	*Platycladus orientalis L*. Treated HeLa Cells
IV	Sorafenib Treated HeLa Cells

RNA Isolation for Gene Expression studies

TRIzol reagent was used to isolate RNA from cells samples treated as above with both compounds, individually and in combination. Further, the RNA samples were quantified by performing spectrometric analysis at 260/280 nm and further examined by performing agarose gel electrophoresis to observe the quality of bands (Ramalho et al. 2004; Puch-Hau et al. 2019).

cDNA Synthesis by RT-PCR and Gene Expression Analysis

The cDNA was synthesized from all the groups of RNA. β-actin an internal control, TIG3, Bcl-2 and PCNA genes were amplified using the respective primers **(Table 2)** and further studied by gel electrophoresis system (UVP gel imaging system) with a 100 bp molecular marker and evaluated using ImageJ software. T100™ Thermal Cycler qRT-PCR system for 40 sequential cycles was used for performing PCR. The total volume was 50 µL, which included 25 µL PCR master mix, 1 µL forward primer, 1 µL reverse primer, 1 µL cDNA template, and 22 µL Milli-Q water used for PCR (Kolenda et al. 2021).

Table 2. Condition applied for gene expression analysis using primers & RT-PCR.

Gene	Forward/ Reverse	Primer Sequences (5′—3′ s)	Product size (bp)
TIG3	F	5′-CAGTATTGTGAGCAGGAACTGTG-3′	84
	R	5′-TTGGCCTTTTCCACCTGTTTAC-3′	
PCNA	F	5′-CCTGCTGGGATATTAGCTCCA-3′	109
	R	5′-CAGCGGTAGGTGTCGAAGC-3′	
Bcl-2	F	5′-ATGTGTGTGGAGAGCGTCAACC-3′	122
	R	5′-GCATCCCAGCCTCCGTTATC-3′	
β-ACTIN	F	5′-AAAGACCTGTACGCCAACACAGTGCTGTCTGG-3′	220
	R	5′-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3′	

Statistical analysis

Experimental studies were run in triplicate and results are expressed as mean ± SD with a P-value ≤ 0.05 are regarded as statistically significant. ImageJ software was used to assess the densities of the gene bands. The IC50 value is calculated using the log(inhibitor) vs. response four-parameter formula.

3. Results and Discussion

Cytotoxicity by MTT assay

In live cells, the enzyme mitochondrial dehydrogenase reacts with MTT to form a formazan crystal. Sorafenib and Platycladus orientalis (L.) leaf extract were found to have IC50 values of 5 µM and 0.6 mg/ml, respectively. The cell samples treated with concentrations 5 µM of Sorafenib showed the viability of 70.16 ± 0.003% and 0.6 mg/ml of Platycladus orientalis (L.), the showed viability of 65.70 ± 0.0026% respectively, whereas the combination of both Sorafenib and Platycladus orientalis (L.) extract in equal ratio showed 40.43 ± 0.0061% of viable cells which is significantly lesser than the control. For Sorafenib treatment, a significant difference was observed between the control and sample concentration ($p \leq 0.001$ for 1, 2, 6, 9, 10 µM and $p \leq 0.0001$ for 3, 4, 5, 7, 8 µM), i.e more toxic than the control's viability percentage and for Platycladus orientalis (L.), a significant difference was observed between the control and sample concentration ($p \leq 0.001$ for 0.15, 0.30, 2.4 mg/ml and $p \leq 0.0001$ for 0.6, 1.2 mg/ml) higher toxic value than the control's viability percentage. The results are given in Figures 1-3.

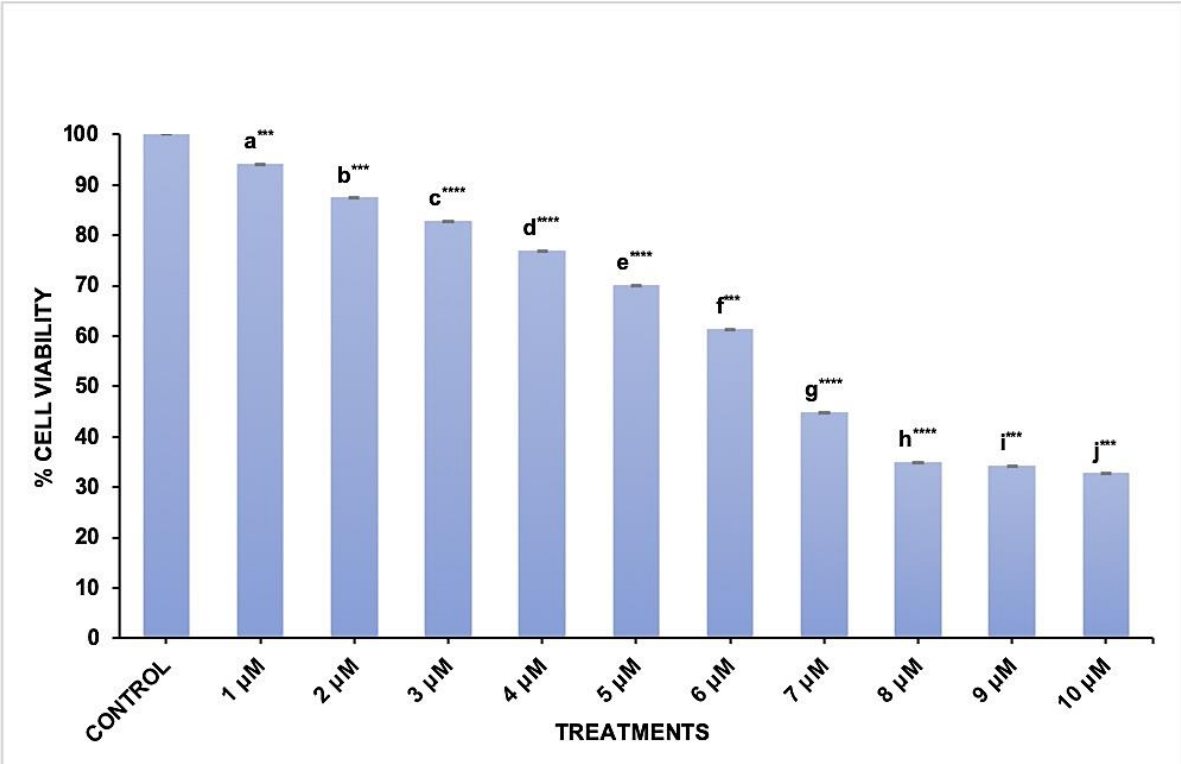

Figure 1. Percentage Cell Viability of HeLa cells post treatment with Sorafenib is a: control vs 1 µM; b: control vs 2 µM; c: control vs 3 µM; d: control vs 4 µM; e: control vs 5 µM; f: control vs 6 µM; g: control vs 7 µM; h: control vs 8 µM; i: control vs 9 µM; j: control vs 10 µM. ***p ≤ 0.001, ****p ≤ 0.0001.

Dye Exclusion assay

The dye exclusion technique was employed to evaluate the cytotoxic effects of the compounds. The trypan blue dye only stains the dead cell and leaves the live-cell unstained due to the impermeable membrane. The percentage of cytotoxicity was calculated for various concentrations of individual treatments and combinations. For Sorafenib 1 µM - 10 µM at 24 h after treatment are, 32.49 ± 10.62%, 36.7 ± 9.82%, 38.83 ± 6.81%, 45.88 ± 3.33%, 48.27 ± 4.14%, 52.79 ± 4.77%, 55.32 ± 4.55%, 56.49 ± 4.46%,

64.17 ± 4.67%, 71.64 ± 4.26% respectively. At 48 h post treatment 1 - 5 µM showed 27.53 ± 5.55%, 34.7 ± 2.88%, 38.5 ± 3.97%, 42.55 ± 4.7%, 45.94 ± 3.33% cytotoxicity and 6 - 10 µM showed 50.12 ± 2%, 53.99 ± 4.66%, 53.82 ± 2.84%, 58.84 ± 2.6%, 68.64 ± 2.23% cytotoxicity respectively and for Platycladus orientalis 0.15, 0.3, 0.6, 1.2 and 2.4 mg/ml at 24 h after treatment are, 32.77 ± 7.67%, 37.38 ± 7.26%, 48.58 ± 2.18%, 56.31 ± 2.71%, 62.13 ± 4.05% respectively. Post 48h treatment 0.15, 0.3 and 0.6mg/ml showed 30.48 ± 6.38%, 35.7 ± 5%, and 45.46 ± 5.52% cytotoxicity and 1.2 and 2.4 mg/ml showed 53.71 ± 6.09%, 57.23 ± 5.59% cytotoxicity respectively. The combination of Sorafenib and Platycladus orientalis i.e 5 µM and 0.6mg/ml concentration at 24 h after treatment showed 80.91 ± 3.95% and at 48 h post treatment showed 78.3 ± 2.74% cytotoxicity. The results are given in Figures 4-6.

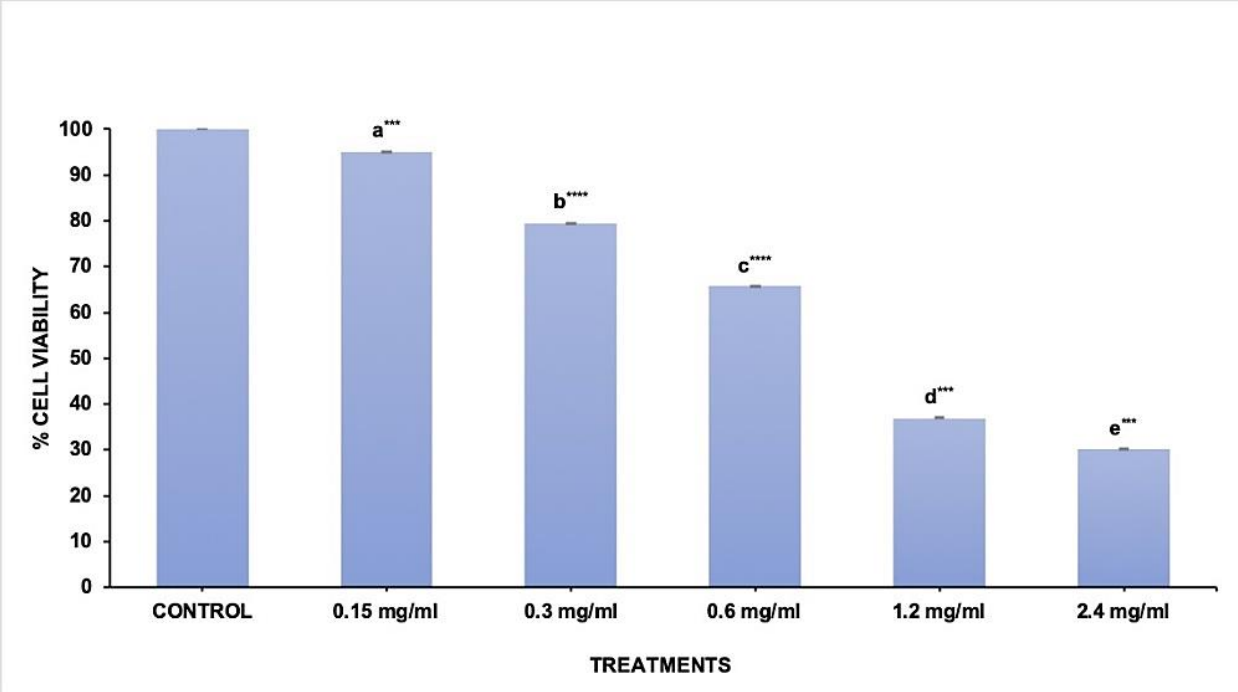

Figure 2. Percentage Cell Viability of HeLa cells post treatment with Platycladus orientalis (L.) is a: control vs 0.15 mg/ml; b: control vs 0.3 mg/ml; c: control vs 0.6 mg/ml; d: control vs 1.2 mg/ml; e: control vs 2.40 mg/ml. ***p ≤ 0.001, ****p ≤ 0.0001.

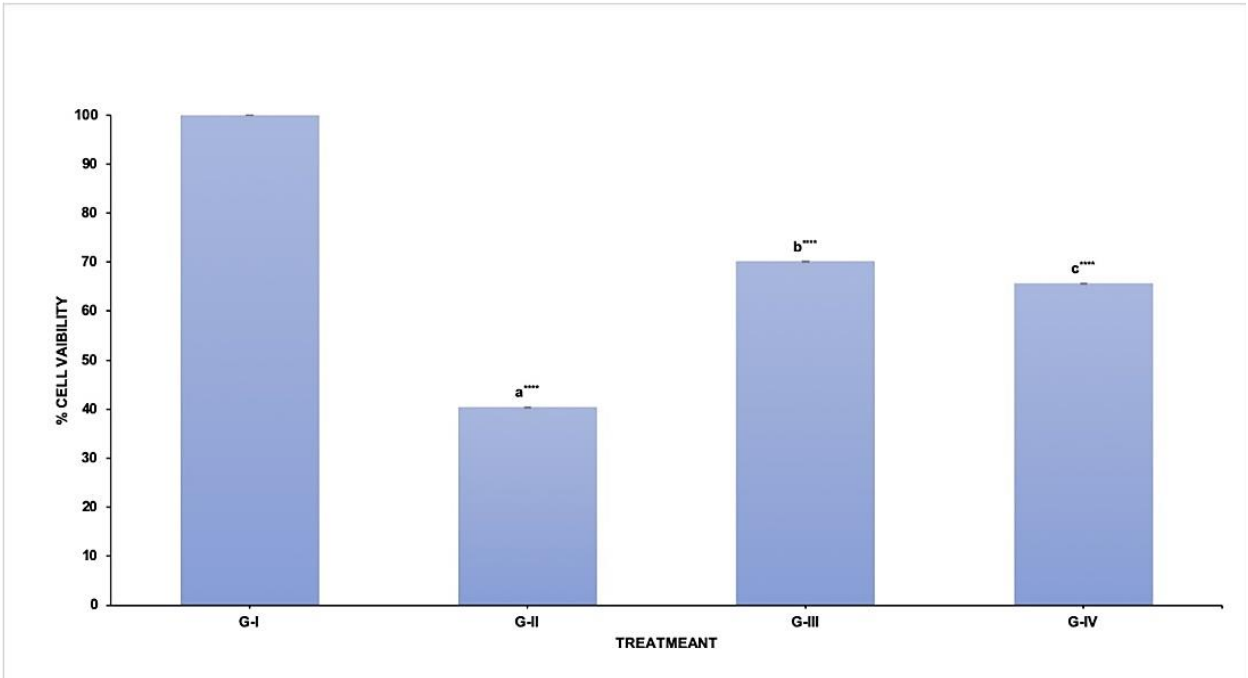

Figure 3. Percentage Cell Viability of HeLa cells post treatment with Sorafenib and Platycladus orientalis (L.) is a: G-I vs II; b: G-I vs III; c: G-I vs IV. G: Group, G-I - Control, G-II - Combination of 5 µM Sorafenib and 0.6 mg/ml Platycladus orientalis L., G-III - 5 µM Sorafenib, G-IV - 0.6 mg/ml Platycladus orientalis L. ***p ≤ 0.001, ****p ≤ 0.0001.

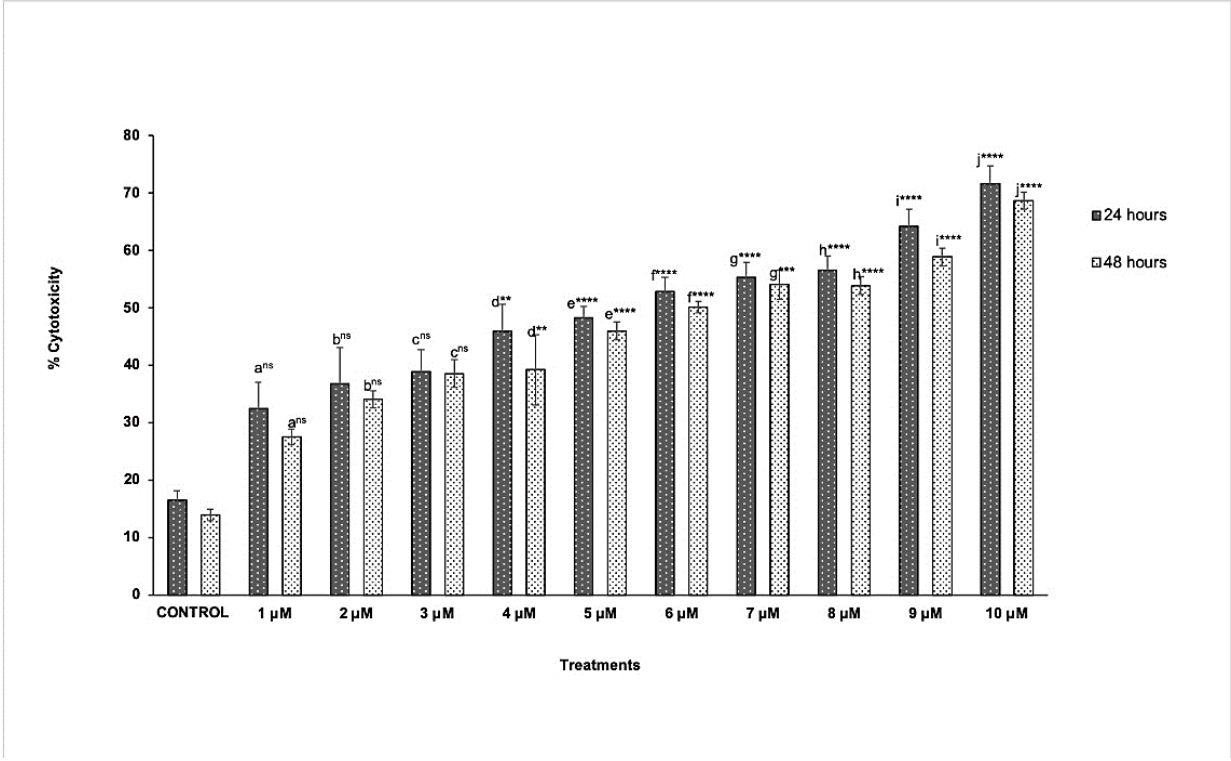

Figure 4. Sorafenib cytotoxicity against HeLa cells at different concentrations. a: control vs 1 µM; b: control vs 2 µM; c: control vs 3 µM; d: control vs 4 µM; e: control vs 5 µM; f: control vs 6 µM; g: control vs 7 µM; h: control vs 8 µM; i: control vs 9 µM; j: control vs 10 µM. Ns: not significant; **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001.

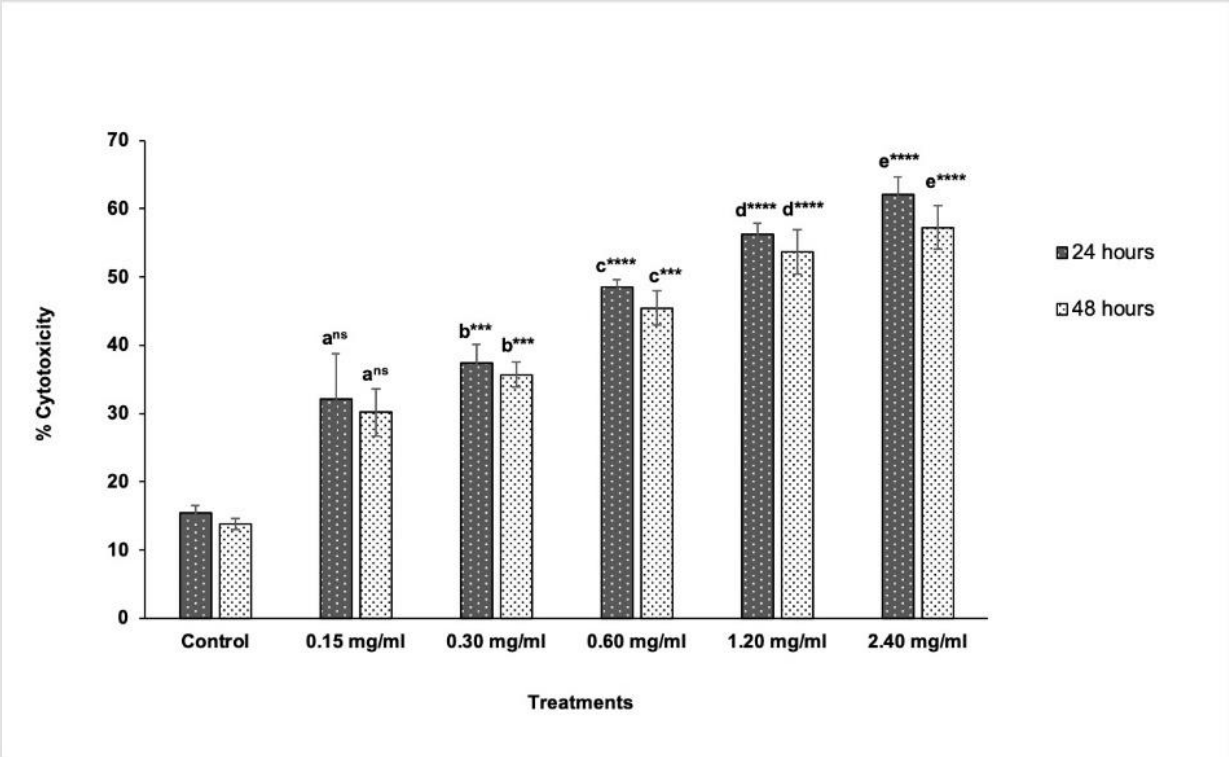

Figure 5. Platycladus orientalis L. extract cytotoxicity against HeLa cells at various concentrations. a: control vs 0.15 mg/ml; b: control vs 0.3 mg/ml; c: control vs 0.6 mg/ml; d: control vs 1.2 mg/ml; e: control vs 2.40 mg/ml. ns: not significant; **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001.

mRNA expression levels of TIG3, PCNA, and Bcl-2

The combined effect of Sorafenib and Platycladus orientalis L. was investigated in this research using HeLa cervical cancer cells by RT-PCR results. The RT-PCR products of the TIG3, PCNA, and Bcl-2 genes were seen as bands with the anticipated molecular sizes of 84, 109, and 122 bp respectively, but at varying amounts depending on the groups (Figure 7A). A very minimal band intensity [Band density (BD)—0.2373] was found in Group I cell sample, whereas Group III and Group IV treated cell samples exhibit high intensity band (BD—0.2490 and 0.2536), respectively, revealing the therapeutic benefit of Sorafenib and Platycladus orientalis L. Leaf extracts. Remarkably, a very elevated band intensity (BD—0.2599) was found in Sorafenib and Platycladus orientalis L. treated Group II samples, revealing that the combinational treatment has notably increased the TIG3 expression level in comparison with the individual treatments (Figure 7B).

Further, a increased intensity band (BD— 0.3276), was found in Group I cell sample and Group III and Group IV treated cell sample showed comparatively low band intensity (BD—0.2603) and (BD—0.2141), respectively. A very low-intensity band (BD—0.1979) was obtained in Sorafenib and Platycladus orientalis L. treated Group II samples showing that the Sorafenib and Platycladus orientalis L. extract has greatly decreased the PCNA expression than the individual treatments (Figure 7C). In addition to this, A high-intensity band (BD—0.3255), was found in Group I cell sample and Group III and Group IV treated cell sample showed low band intensity (BD—0.2587 and 0.2157), respectively. A very low-intensity band (BD—0.1998) was obtained in the combination of Sorafenib and Platycladus orientalis L. treated Group II samples showing that the Sorafenib and Platycladus Orientalis L. extract has greatly decreased the Bcl-2 expression than the individual treatments (Figure 7D).

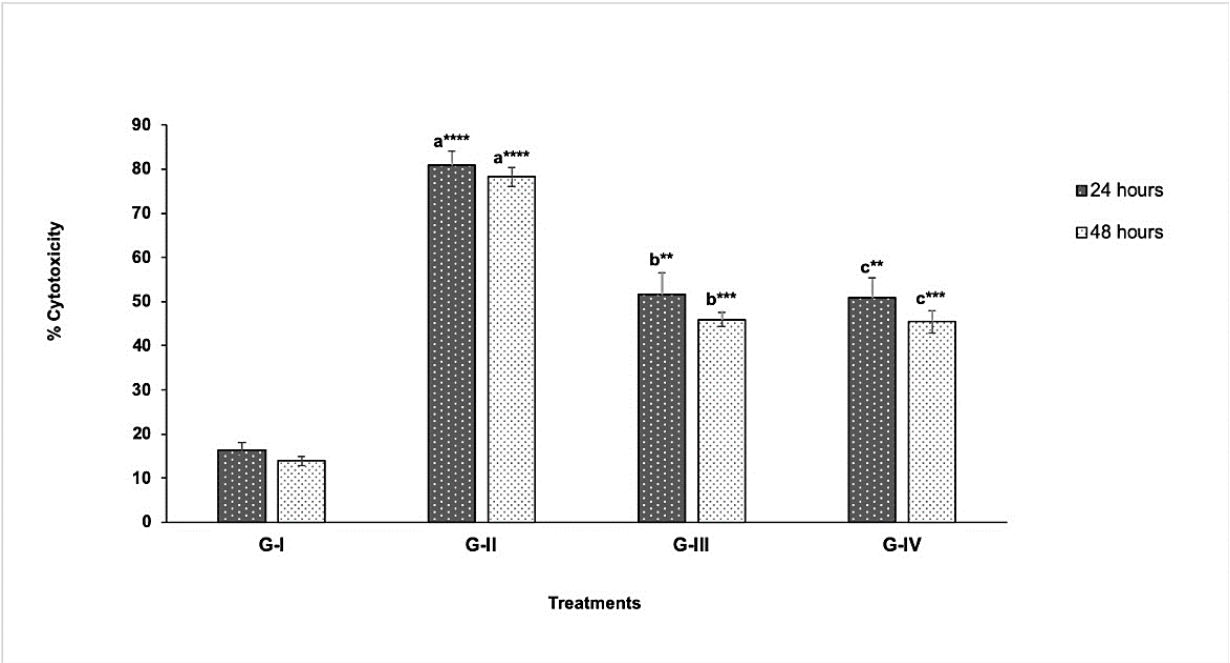

Figure 6. Cytotoxicity of various combination and individual concentrations with Sorafenib and Platycladus orientalis (L.) extract against HeLa cells. (a: G-I vs II; b: G-I vs III; c: G-I vs IV. G: Group), ns: not significant; **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, G-I: Control, G-II: Combination of 5 µM Sorafenib and 0.6 mg/ml Platycladus orientalis L., G-III: 5 µM Sorafenib, G-IV: 0.6 mg/ml Platycladus orientalis L.

Despite of latest tools and techniques for understanding the molecular mechanisms of the development of human cervical cancer and in the development of treatment strategies, cervical cancer still showing the prominent causes of cancer death worldwide (Hussain et al. 2003). According to a World Health Organization (WHO) study, up to 80% of cervical, lung, breast, and other malignant cells have overexpression or mutation of the EGFR signaling pathway (Marchetti et al. 2005) and this plays a crucial role in maintaining regular life activities (Tiseo et al. 2010; Ren et al. 2015; Tu et al. 2018). The major downstream EGFR signaling pathways reported in the literature are the PI3K/Akt/mTOR pathway, the Ras-

Raf-MARK pathway, and the JAK-STAT pathway (Jiang and Liu 2009; Courtney et al. 2010). Various activities such as cell survival, proliferation, and differentiation are jointly controlled by the coordination of these signaling pathways. (Vivanco and Sawyers 2002; OuYang et al. 2018). The main cause of cervical cancer was considered to be the disorder in PI3K/mTOR signal pathway (Roden and Wu 2006). Sorafenib is a multi-kinase inhibitor drug, which acted against these activated kinases (Wilhelm et al. 2008; Smalley et al. 2009; Keating and Santoro 2009) and its treatment has induced autophagy (Zhang et al. 2014), which lead to tumor growth suppression. The anti-cancer molecular action of sorafenib thus exhibits the suppression of human cancer cell proliferation as well as induces cell death mechanism via regulating the above-mentioned signaling pathways. In this study, we have evaluated the treatment effects of the combination of sorafenib and Platycladus orientalis L. leaf extract on the expression levels of proliferation marker, PCNA, tumor suppressor, TIG3, and apoptosis suppressor, Bcl-2.

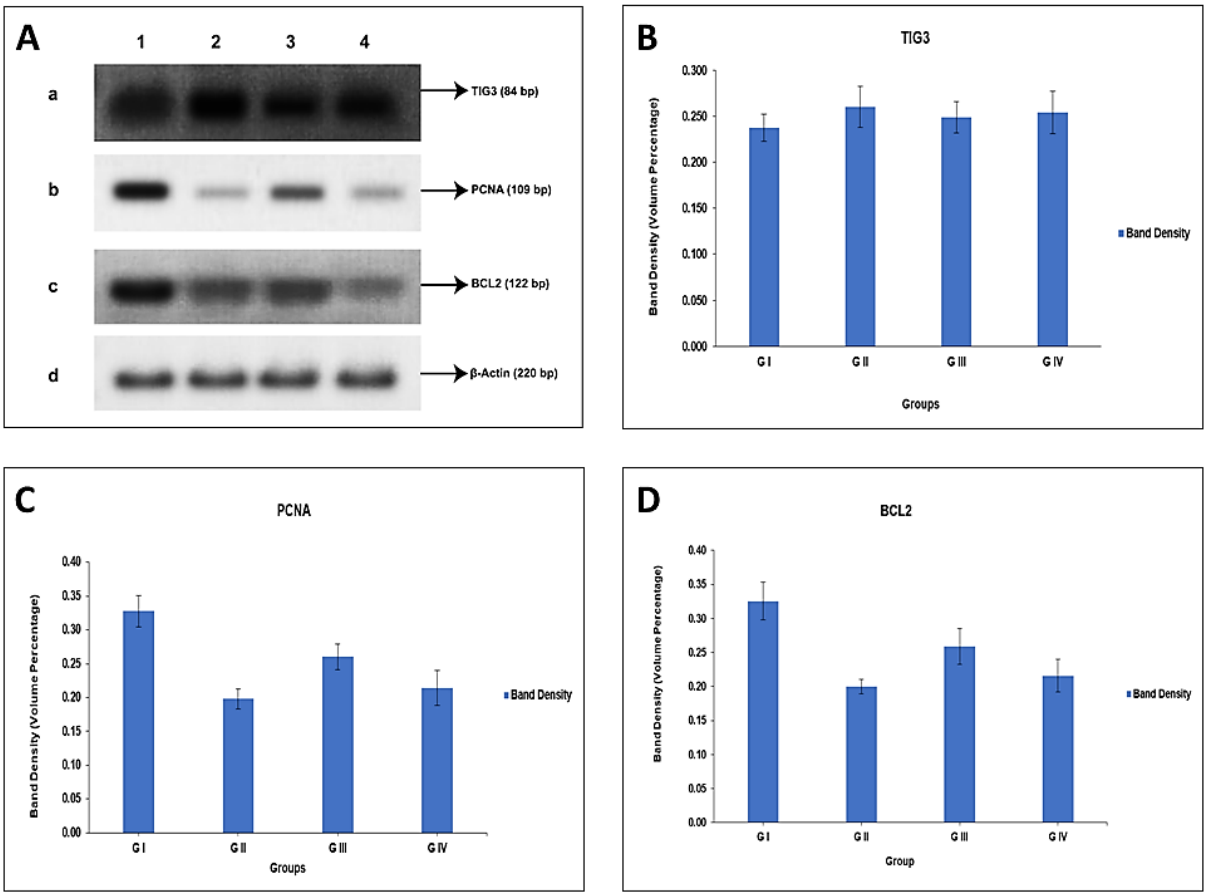

Figure 7. Expression of TIG3, Bcl-2, and PCNA. Lane 1: Cancer control sample (Group I). Lane 2: cancer cells treated with the combination of Sorafenib and Platycladus orientalis L. (Group II). Lane 3: cancer cells treated with Sorafenib (Group III). Lane 4: cancer cells treated with Platycladus orientalis L. (Group IV) (A). The densitometric plot of TIG3 bands (B). The densitometric plot of PCNA bands (C). The densitometric plot of Bcl-2 bands (D).

The synergetic effect of sorafenib and Platycladus orientalis L. on cervical cancer treatment efficiency might have been resulted by targeting TIG3, and an enhanced TIG3 mRNA has been proven in suppressing the RAS protein which is activated by Ras/MAPK and other signalling molecules in cervical cancer cells (Huang et al. 2002; Tsai et al. 2006), thereby decreasing the growth of cells. Besides, The Bcl-2 family which consists of pro-apoptotic proteins that are activated by the intrinsic apoptotic pathway by oligomerization, mediates the release of ROS and Cyt-C from the mitochondrial intermembrane space. Cyt-C on interaction with the Apaf-1 in the cytoplasm, leads to the formation of apoptosome that recruits and activates pro-caspase 9 and thereby induces a caspase cascade that leads to apoptosis (Finucane et al. 1999). The downregulations of Bcl-2 expression level leads to cell cycle arrest and apoptosis (Vaux et al. 1988; Uren et al. 2007; Fletcher et al. 2008). The PCNA is a crucial component of the cell's DNA replication and repair cycle (Chatterjee et al. 2019). The expression of HPV E7 oncoprotein that is responsible for

reverting the function of p21 (Cip1)-mediated inhibition of PCNA in HPV immortalized cervical cancer cells was found to be downregulated by certain drugs and shown negative regulation in cell growth (Branca et al. 2007).

The findings of this study suggest that Platycladus orientalis L. and sorafenib together are a powerful inhibitor of cell growth and a robust inducer of apoptosis. The sorafenib or Platycladus orientalis L. concentration of half-maximal anti-neoplastic effects (IC_{50}) was approximately 5 µM and 0.6 mg/ml, respectively. In current manuscript, we have also found a drastic increase in the level of tumor suppressor TIG3 expression and reduction of PCNA and Bcl-2 expression in the human cervical cancer cells when compared with the control. Accordingly, the study demonstrated that sorafenib and Platycladus orientalis L. have suppressed the cell growth in a dose-dependent way, with the combined therapy displaying stronger anti-proliferative effect.

4. Conclusions

The overall results show that the combination therapy of sorafenib and Platycladus orientalis L. extract in an equal ratio of 5 µM and 0.6 mg/ml respectively has the potential to impede the proliferation of HeLa cells. Furthermore, the mRNA expressions of PCNA, Bcl-2 & TIG3 were regularized effectively by the combination therapy than the individual drugs have done. Thus, the combination therapy of sorafenib and the extract of Platycladus orientalis L. has favorable characteristics for anticancer therapy. The future impact of the study is to explore the possibility of enhancing the treatment efficiency of chemotherapeutic agents, Sorafenib on HPV associated cervical cancer by combinational treatment with phytochemical extracts of Platycladus orientalis (L), having a natural anti-viral as well as anti-oxidant potencies, which may reduce the toxic side effects too. However, extensive molecular research and pre-clinical animal experiments are required to have a better comprehension of the mechanism underlying the anti-cancer improvement of the specific combination, as well as its cytotoxicity *in-vivo* for therapeutic purpose.

Authors' Contributions: SAMUEL, E.: data acquisition, analysis and interpretation of data, drafting the article; WILSON, D.D.: drafting the article, editing, plagiarism and grammar check; TOMY, C.: data acquisition, analysis and interpretation of data, drafting the article; NALLAKANNU, D.J.I.: data acquisition, analysis and interpretation of data, drafting the article; SIDDIKUZAMAN.: analysis and interpretation of data, drafting the article; AVANY, B.: data acquisition, analysis and interpretation of data; GRACE, V.M.B.: conception and design, analysis and interpretation of data, drafting the article. All authors have read and approved the final version of the manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: We would like to extend their gratefulness to the Karunya Institute of Technology and Sciences, Coimbatore, India for research facilities and the Nano-therapy Research Lab, supported by DST, GOI [SB/YS/LS-252/2013(15 May 2014)] and DBT, GOI [BT/PR14632/NNT/28/824/2015].

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Received: 28 July 2021 | **Accepted:** 7 June 2022 | **Published:** 27 January 2023

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