Research Article

Apoptosis Activity of *Ruellia tuberosa* Leaf Extract on HUH-7, SW480 & HEK293T Cells

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Received: 14-10-2021; **Revised:** 22-12-2021; **Accepted:** 30-12-2021; **Published on:** 15-01-2022.

ABSTRACT

The plant *Ruellia tuberosa* is a traditional medicine as a broad-spectrum medicine. *Ruellia tuberosa* leaf extract (RTLE) showed potential anti-cancer activity in our previous study on HepG2 cells. RTLE significantly inhibited the cell viability in a time and concentration dependent manner on the Huh-7 and SW480 cell lines and being a normal human cell line there was insignificant inhibition in Hek293T cell line. Cell images clearly showed nuclear disintegration, several signs of apoptosis like chromatin condensation, nuclear fragmentation and formation of apoptotic bodies whereas the untreated control cells were with intact nuclei when treated with RTLE on (Huh-7) hepatocellular carcinoma cells. Flow cytometric analysis confirmed the presence of apoptotic cells in the early and late apoptotic stages of Huh-7 and SW480 cells but not in Hek293T. Cell cycle phase arrest was observed in the G0/G1 phase in Huh-7 and SW480 cells but not in Hek293T cells. MMP shift assay exhibited significant change in the RTLE treated Huh-7 and SW480cells but no such shift was observed in Hek293T cells. These findings suggest that RTLE mediates cytotoxicity in hepatocellular and colon carcinoma cells via apoptosis without exerting any such toxic effect on normal human embryonic kidney cells. Further mechanistic studies are going on to determine the different pathways contributing to apoptosis.

Keywords: *Ruellia tuberosa* leaf, Hepatocellular carcinoma, Colorectal carcinoma, Apoptosis.

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DOI:
10.47583/ijpsrr.2022.v72i01.007

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v72i01.007

INTRODUCTION

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Mutations in genes can also cause cancer by accelerating cell division rates or inhibiting normal controls on the system, such as cell cycle arrest or programmed cell death.[1] Liver and Colon are the major organs of human body. The liver is the largest gland in the body and performs an astonishingly large number of tasks that impact all body systems. Hepatocellular carcinoma (HCC), or hepatoma accounts for more than 90% of all cases of primary liver cancer.[2] It is the sixth most common type of cancer worldwide and has shown a significant increase in its incidence, becoming third leading cause of cancer-related mortality.[3] Colorectal cancers is one of the most common cancer in people over 50 years both in the developed and less developed world. The types of cells that most commonly give rise to colorectal cancers is the adenocarcinoma which arise from inner line of the inside tissue of colon and rectum. Treatment efficiency of colorectal and liver cancer are very low, therefore colorectal and liver cancer prevention and control is extremely important. Drug discovery from medicinal plants has played a very important role in the treatment of cancer. *Ruellia tuberosa* was used as a cooling agent in urinary problem and also used for treatment of uterine fibroids. It has been recently incorporated as a component in an herbal drink in Taiwan. In Siddha system of medicine, leaves are given with liquid copal as remedy for gonorrhea and ear diseases.[4] The *Ruellia tuberosa* leaf extract and its constituents have been reported to possess anti-cancer activity against EAC cells.[5] In our previous work we have studied on hepatocellular carcinoma (HepG2) cell line. HepG2 is a hepatocellular carcinoma originated from 15-year-old American male Caucasian.[6] But, no such work has been reported on another very well-known hepatocellular carcinoma cell line i.e. Huh-7, a 57-year-old Japanese origin hepatocellular carcinoma cell line. Therefore, we evaluated the anti-proliferative activity of *Ruellia tuberosa* leaf extract (RTLE) using Huh-7hepatocellular carcinoma cell line. Due to high mortality rate of colorectal carcinoma we have studied the effect of RTLE on SW480 cell line, a colorectal cancer line which is a 50 year male Caucasian. The toxic effect of *Ruellia tuberosa* leaf extract on HEK293T cell line was also investigated.

MATERIALS AND METHODS

Chemicals

DMEM (Gibco), fetal bovine serum(FBS), Trypsin (Gibco, USA), Penicillin-streptomycin (Biowest, Germany), Gentamycin (Nicholas, India), HEPES, L-glutamine, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)], DMSO (dimethylsulphoxide), Acridine orange, Ethidium bromide, ethylene diamine tetra acetic acid

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(EDTA) Agarose (Puregene), Proteinase k (SRL), RNAse, JC-1 dye, Propidium iodide (Sigma), Chloroform, isoamyl alcohol, Methanol (Merk), and all other chemicals and reagents were of analytical grade and procured locally.

Cell culture

Huh-7 was obtained from Nation Centre for Cell Science, Pune, India. The cells were cultured and routinely maintained in DMEM medium and the medium was supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100µg/ml), gentamycin (100µg/ml) and were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 inside a CO_2 incubator. All cell lines were adherent in nature.

Collection, Identification, Extraction and preparation of test samples

The leaves of *Ruellia tuberosa* were collected from the nursery of Ramakrishna Mission Ashram, Narendrapur. The plant was identified by Dr. K. Karthigeyan, scientist C, Central National Herbarium, Indian Botanical Garden, Howrah, India. Leaves of *Ruellia tuberosa* were collected, shade dried and grinded into fine dust.200gm of *Ruellia tuberosa* leaf powder was taken in a conical flask and soaked in 500ml hexane for 3 days with occasionally shaking for removal of fat. After 3 days, the mixture was filtered and the filtrate was evaporated by Rotary evaporator. After evaporation 2gm of hexane extract was obtained and was stored in air tight container. After fat removal from *Ruellia tuberosa* leaves, it was soaked in 3x500 ml of methanol for one week with occasional shaking. The mixture was filtered and the filtrate was evaporated by Rotary evaporator. After evaporation 3.4gm of sticky methanolic extract was obtained finally and designated as RTLE and kept in air tight container at 4° C. Stock solution was prepared as 1mg/ml in PBS from here desired concentrations (25,50,100,200 µg/ml) was used for *in-vitro* experiments.

Cytotoxicity study by MTT assay

Huh-7, SW480, HEK293T cells (1x10^{5}) were seeded in 96-well sterile plates and were treated with different concentrations (25,50, 100,200 µg/ml) of RTLE for 24, 48 and 72 hrs.[7] The treated cells were grown in humidified atmosphere containing 5% CO_2 in an incubator at 37°C and the untreated cells were considered as control. After 24, 48 and 72 hrs incubation 20µl of MTT (4-5mg/ml in PBS as a stock solution) was added to each well and incubated again for 3 to 4hrs at 37°C. MTT assay is a colorimetric assay for assessing the metabolic activity of the cells or cell viability of NAD(P)H dependent cellular oxido-reductase enzymes, and represents number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT, which is yellow in colour, to insoluble purple colored formazan. The intensity of the colour was measured at 570 nm by micro-plate manager (Reader type: Model 680 XR Bio-Rad Laboratories Inc.).The IC_{50} values were determined for the cells.

Detection of cell morphological by Fluorescence Microscope

Huh-7 cells (1x10^{6}) was treated with $\mathbf{IC_{50}}$ of RTLE for 24 h were observed using a fluorescence microscope for morphological changes. The untreated control cells and the RTLE treated cells were harvested separately, washed with PBS and then stained with acridine orange (100 µg/ml) and ethidium bromide (100 µg/ml) (1:1). The cells were then immediately mounted on slides and observed under a fluorescence microscope in (Olympus, Fluoview FV10i) at 60x. for the morphological determination of the cells undergoing apoptosis.

Detection of cell morphology by Confocal Microscope

Huh-7 cells(1x10^{6}) was treated with $IC_{50}$of RTLE for 24 h. After 24 hrs the untreated control cells and RTLE treated cells were harvested and washed with ice cold PBS. The cells were then stained with 10 µg/ml of propidium iodide separately for 5 min. After mounting on slides the cells were observed to see the differences in nuclear morphology between the untreated and the RTLE treated cells under confocal laser scanning microscope (Olympus, Fluoview FV10i) at 60x. Images for propidium iodide were acquired from argon/krypton laser and UV laser line using 590 nm long pass filter for propidium iodide and 450 nm band pass filter for UV images.

Detection of DNA Fragmentation by Agarose Gel Electrophoresis

Huh-7, SW480 cells (1x10^{6}) were treated with IC_{50} dose but Hek293T cells treated with 100µg/ml dose of RTLE for 24 h The cells were re-suspended in 500 µl of lysis buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 0.5% SDS), 100 µg/ml of proteinase K was added and incubation was done at 55 °C for 1 h and 37 °C overnight respectively.[8] DNA extraction was done by following the general phenol-chloroform extraction procedure and kept at -20 °C overnight. After centrifugation, DNA precipitates were washed with 70% chilled ethanol, dried and evaporated at room temperature and dissolved in TAE buffer (pH 8.0) at 4 °C overnight. To detect the DNA fragments, the isolated DNA samples were electrophoresed overnight at 20 V in 1% agarose gel and stained with ethidium bromide. DNA fragmentation was observed in UV transilluminator. (GENEI, Bangalore Genei Pvt. Ltd.)

Detection of mitochondrial membrane potential (Δψm) assay

Huh-7, SW480, Hek293T (1x10^{6}) cells were treated with RTLE with desired dose and untreated as control for 24 hours to assay the mitochondrial membrane potential activity of cell in a flow cytometry. Cell were washed with PBS, pelleted down and eventually stained with JC-1 stain. The sample were incubated at 37ºC for 15 min. Shift in the mitochondrial membrane potential was determined by FACS (Becton Dickinson FACS Fortessa 4 leaser cytometer), Florence detector equipped with 520 nm argon laser light

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source and 623 nm band pass filter (liner scale) with the help of BD FACS Diva software (Becton Dickinson).

Detection of Apoptosis by Flow Cytometric analysis

In order to investigate the type of cell death induced by RTLE, flow cytometric analysis was done by performing dot plot assay. Huh-7, SW480, Hek293T ($1x10^6$) were treated with desired dose of RTLE for 24 h.[6] The cells were pelleted down, centrifuged at 2000 rpm for 8 min at 4 °C and washed with Annexin V FITC binding buffer (10 mM HEPES, 140 mM NaCl and 2.5 mM CaCl2 2H2O; pH 7.4). Again after centrifuging at 2000 rpm at 4 °C, the cell pellets were dissolved in Annexin V FITC binding buffer containing annexin V FITC and propidium iodide. After 15 min incubation in dark at room temperature flow cytometric analysis was done. All data were acquired with a Becton-Dickinson FACS Caliber single laser cytometer. Flow-cytometric reading was taken using 488 nm excitation and band pass filters of 530/30 nm (for FITC detection) and 585/42 nm (for PI detection). Live statistics were used to align the *X* and *Y* mean values of the Annexin-V FITC or PI stained quadrant populations by compensation. Data analysis was performed with Cell Quest (Macintosh platform) program.

Detection of Cell Cycle Arrest by Flow Cytometric Analysis

In order to study the stage of cell cycle arrest in flow cytometry Huh-7, SW480 & Hek293T cells ($1x10^6$) were treated with desired of RTLE for 18 h. Cells were washed with PBS, fixed with cold methanol by adding methanol drop-wise and kept at -20 °C for 3 min. They were then resuspended in cold PBS and kept at 4 °C for 90 min. Cells were pelleted down, dissolved in cold PBS, treated with RNase A for 30 min at 37 °C and stained with propidium iodide (20 µl from 50 µg /ml) and kept in dark for 15 min. Cell cycle phase distribution of nuclear DNA was determined on BD FACS Diva software (Becton Dickinson FACS).[9]

Statistical Analysis

Percentage of cell growth inhibition was calculated by the following formula: % Cell Inhibition= 10 X (O.D of Control – O.D. Of treated /O. D. of Control), O. D= Optical Density.

Percentage of cell viability was calculated as follows: Viable Cells (%) = (Total number viable cells per ml/Total number of cells per 1ml) x100.

RESULTS

Cytotoxicity study by MTT assay

In the MTT assay, Huh-7, SW480, cells there was significant reduction in the O.D values after treating all the, the with RTLE in a time and concentration dependent manner compared to that of control cells whereas in HEK293T cells insignificant reduction in the O.D values were observed. These observations provided proof for cytotoxic nature of RTLE towards only on all the carcinoma cell lines. The IC_{50} value of RTLE was 44.97µg for Huh-7 cells, 41.6µg for SW480 respectively.

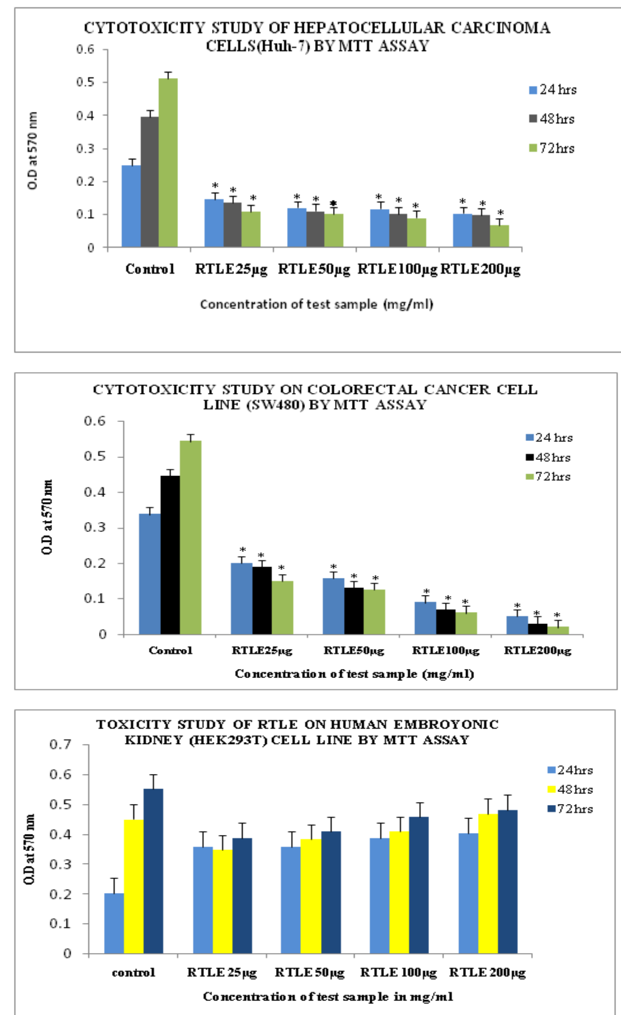

Figure 1: Histogram Showing the O.D. values of Huh-7, SW480, Hek293T cells treated with RTLE.RTLE treated all the carcinoma cells showed significant reduction in O.D. value in a time and concentration dependent manner whereas in normal cells showed insignificant reduction in the O.D. Values up to 72 hrs. Data are mean ± S.E.M.

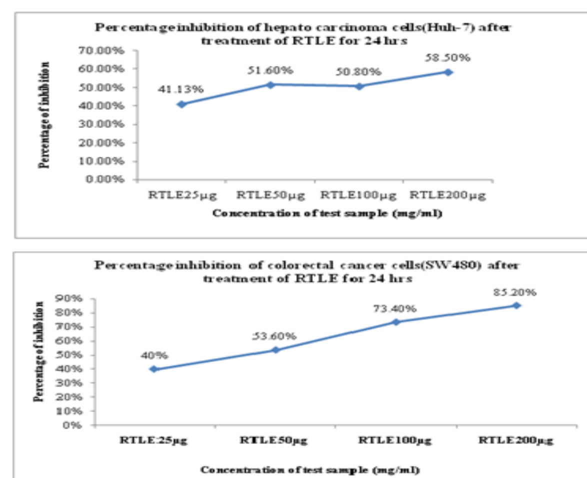

Figure 2: Line graph shows the percentage inhibition of viable cells in Huh-7, SW480 cells treated with RTLE for

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24hrs. RTLE treated cells significantly increases the % inhibition in a time and concentration dependent manner. The IC_{50} value of RTLE were calculated for Huh-7 44.97µg, SW480 41.6µg for 24hrs respectively.

Detection of cell morphology by Fluorescence & Confocal Microscope

Observations revealed that RTLE treated and control Huh-7 cells was stained with both acridine orange and ethidium bromide confirm the presence of apoptotic cells early and late as compared to the untreated control cells. In this study nuclear changes were observed including chromatin condensation and apoptotic body formation that are the indication of apoptotic processes. Apoptosis further confirmed by confocal images of RTLE induced apoptotic changes in Huh-7cells after 24 h of treatment showing chromatin disintegration and formation of apoptotic bodies whereas the untreated control cells were with intact nucleus.

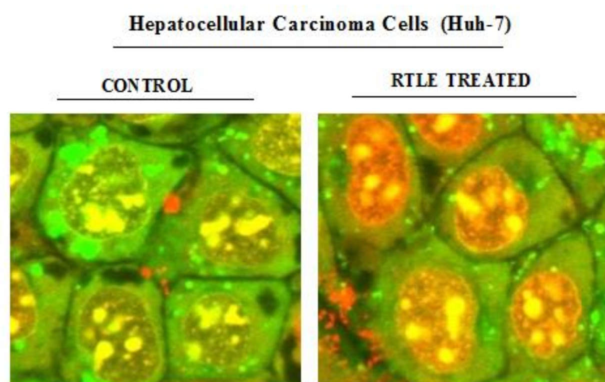

Figure 3: Fluorescence and confocal microscopic images of both the untreated control and RTLE treated hepatocellular carcinoma cells. Control cells give a bright green fluorescence whereas the treated cells show an orange red colour, demarking the occurrence of Apoptosis in hepatocellular carcinoma, Huh-7 cells.

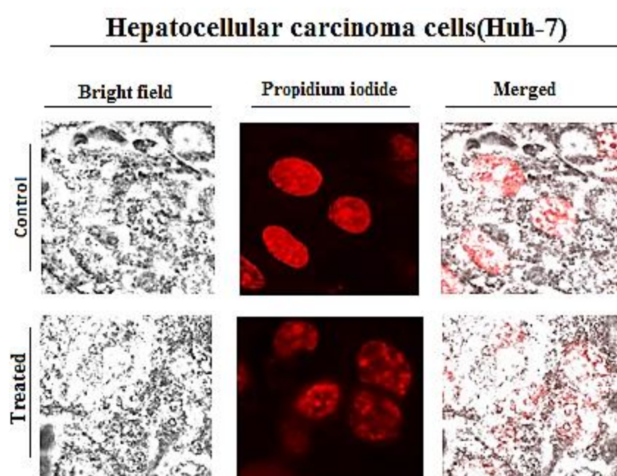

Figure 4: Confocal microscopic image of Huh-7 cell line after 24 hours of treatment with RTLE. Treated cells showed nuclear fragmentation which is a sign of apoptosis whereas control cells showed intact nucleus which has been indicated by arrow.

Detection of DNA Fragmentation by Agarose Gel Electrophoresis

The gel pattern of the DNA samples isolated from untreated control Huh-7, SW480 cells showed intact DNA bands, whereas the gel pattern of the DNA samples isolated from RTLE treated Huh-7 and SW480 cells showed degraded DNA bands in the form of ladders. So, the observations confirmed that the treatment with RTLE in both the cells caused apoptosis.

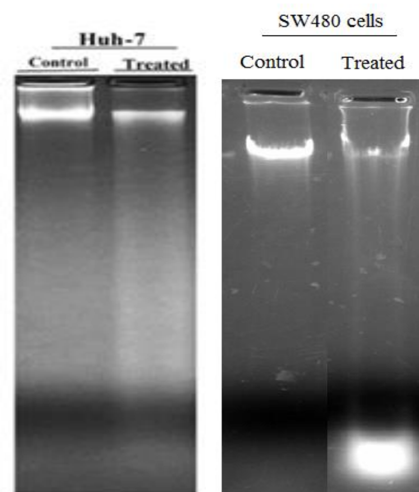

Figure 5: DNA fragmentation by agarose gel electrophoresis in Huh-7 and SW480cells. Lane 1 represent control cells which shows intact DNA whereas Lane 2 represent RTLE desired dose treated cells in which DNA fragments are clearly visible in carcinoma cells.

Detection of mitochondrial membrane potential (Δψm) assay

Mitochondrial dysfunction is an essential target for induction of apoptosis. The hepatocellular carcinoma (Huh-7), colorectal cancer (SW480) and normal(Hek293T) cell lines when treated with desired dose of RTLE, there was a loss of Mitochondrial Membrane Potential (Δψm) of cancer line and insignificant loss in normal cell line. The JC-1 stain cannot accumulate in the mitochondria of the apoptotic cells, as the mitochondrial membrane potential collapses, hence showing green fluorescence (P4) denotes apoptotic cells and red fluorescence (P3) denoting healthy cells where JC-1 stain accumulates. Depolarization in mitochondrial membrane potential was observed by staining untreated and treated cell by JC-1 dye. The depolarization led to a transmembrane shift from red to green fluorescence leading to release of cytochrome c. A significant transmembrane shift of 9.6% to 41.7%,12.4% to 58.5% were observed when Huh-7, SW480 cells were treated with the IC_{50} value of RTLE for 24 hours respectively. But an insignificant transmembrane shift of 14.6% to 9 % were observed when HEK293T was treated with the desired dose of RTLE for 24 hours.

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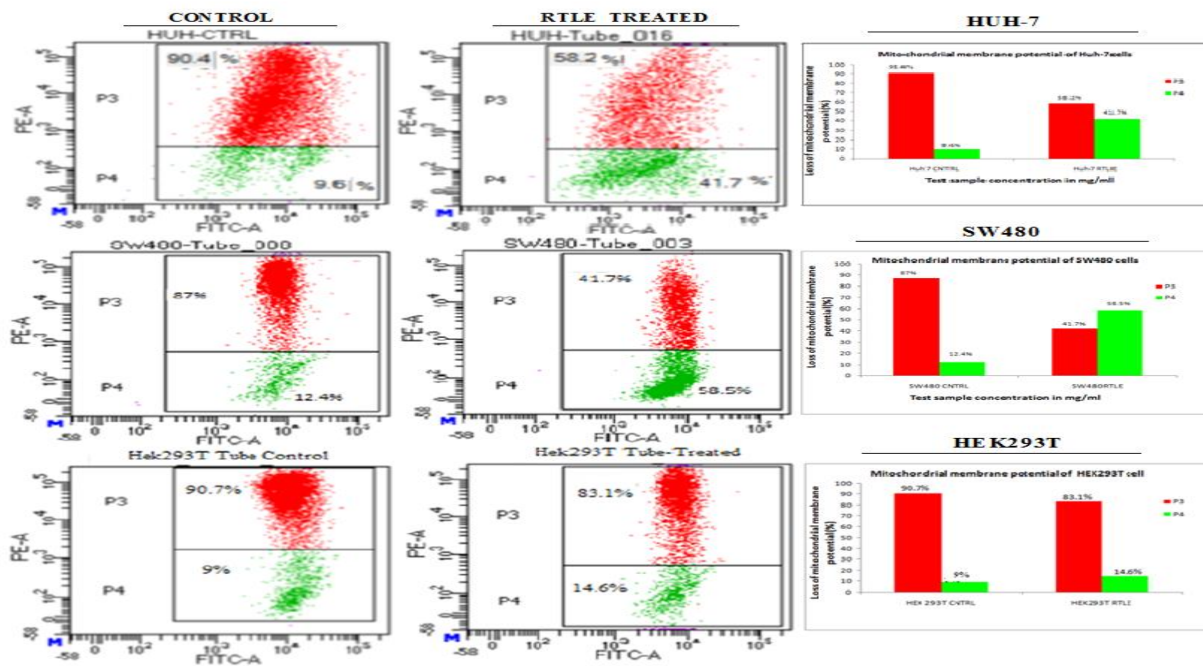

Figure 6: Flow cytometric analysis of mitochondrial membrane potential (Δψm) on Huh-7, SW480 and HEK293T cell lines in both control and RTLE treated cells respectively after 24 hrs of treatment. Transmembrane shift was significantly increased in Huh-7, SW480 cells whereas in the normal HEK 293T cells insignificant increase observed when treated with desired dose of RTLE. Histogram showing the fold's decrease in mitochondrial membrane potential (Δψm) in Huh-7 and SW480 cells and increase in mitochondrial membrane potential(Δψm) in HEK 293T cells when treated with RTLE.

Detection of apoptosis by flow cytometry

In the flow cytometric analysis, double labeling technique, using Annexin V-FITC and propidium iodide, was utilized. Lower left (LL) quadrant (Annexin V-/PI-) is regarded as the population of live cells, lower right quadrant (LR) (Annexin V+/PI-) is considered as the cell population at early apoptotic stage, upper right (UR)quadrant (annexin V+/PI+) represents the cell population at late apoptotic stage. Flow cytometric data analysis revealed that after 18h of treatment with desired dose of RTLE for quantification of apoptosis was observed for Huh-7 1.2% against 22.3%, SW480 0% against 25%, cells were in upper right quadrant which implies apoptotic cells thereby, showing apoptotic inducing property of RTLE on colorectal and hepatoma cells. In normal cells HEK293T 80.5% against 80.9% cells were in upper right quadrant does not implies apoptotic cells thereby, RTLE shows non-apoptotic inducing property towards both the normal cell lines.

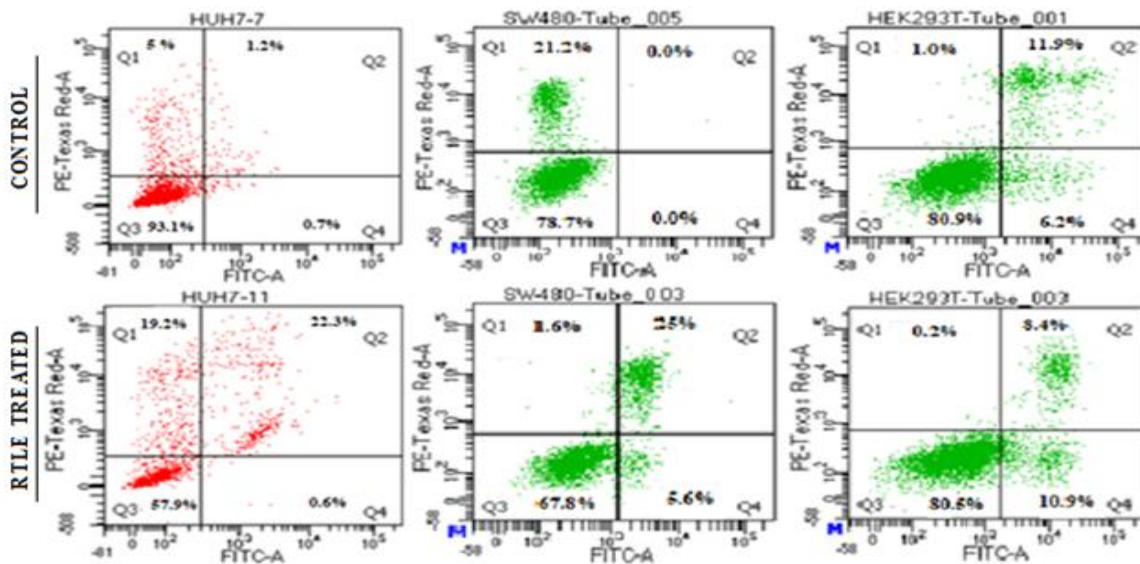

Figure 7: Flow cytometric analysis of untreated control and RTLE treated of Huh-7, Sw480 and HEK 293T cells respectively stained with Annexin V FITC and propidium iodide. Dual parameter dot plot of FITC fluorescence (*x*-axis) *vs* PI-fluorescence (*y*-axis) shows logarithmic intensity.

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Detection of Cell Cycle Arrest by Flow Cytometric Analysis

Flow cytometry analysis showed that after 24hrs treatment of Huh-7, SW480 cell lines with RTLE at desired dose, sub-G1 peak was markedly changed, but in case of Hek293T cells insignificant change at sub-G1 peak was observed. The DNA content increase in RTLE treated cell 56% against 37.9% in Huh-7, 32.2% against 30% in SW480, 70.9% against 71% in HEK293T cells. These observations revealed that RTLE significantly inhibited the growth of Huh-7, SW480, cells by arresting the cell populations in the sub-G0/G1 phase of the cell cycle.

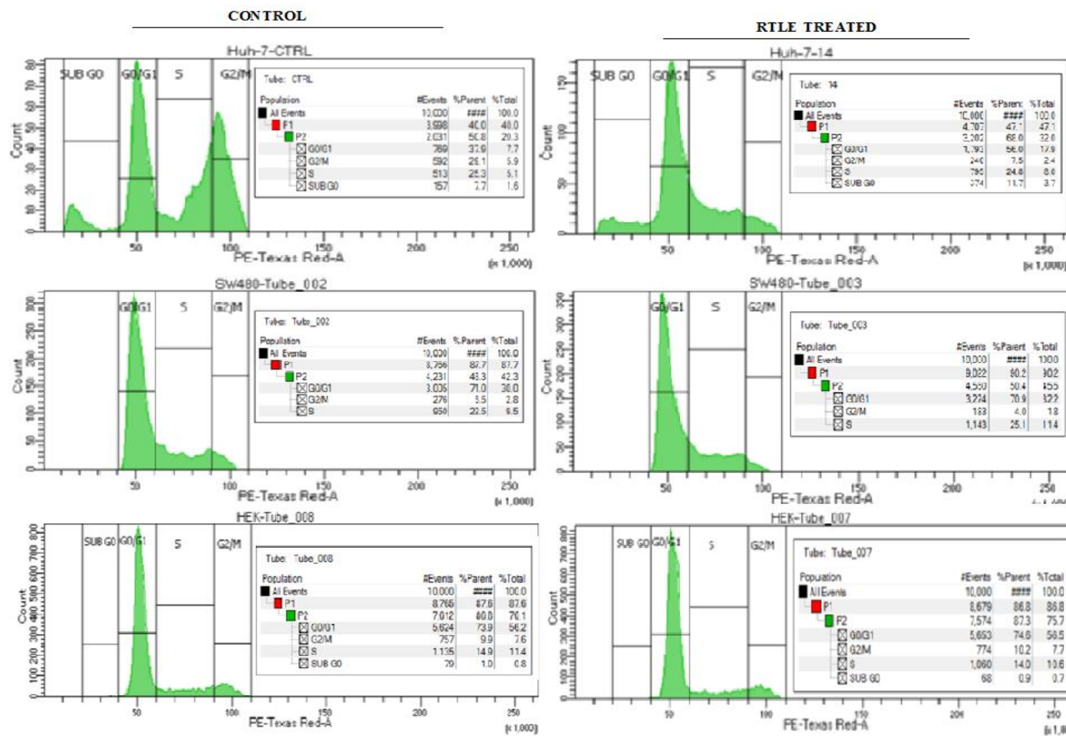

Figure 8: Flow cytometric analysis of cell cycle phase distribution in control and treated Huh-7, SW480, Hek293T cells respectively after 18 hrs treatment at desired dose of RTLE.

DISCUSSION

Hepatocellular carcinoma (HCC), or hepatoma accounts for more than 90% of all cases of primary liver cancer and colorectal cancer is one of the most common cancer in people over 50 years both in the developed and developing countries. Primary liver cancer includes hepatocellular carcinoma (HCC) (comprising 75%-85% of cases) and intrahepatic cholangiocarcinoma (comprising 10%-15% of cases) as well as other rare types. The main risk factors for HCC are chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), aflatoxin-contaminated foodstuffs, heavy alcohol intake, obesity, smoking, and type 2 diabetes.[10] The rising obesity prevalence is considered a contributory factor to the observed increasing incidence of HCC in low-risk HCC areas.[11] Primary prevention of the majority of liver cancer cases has been feasible through a vaccine against HBV since 1982, and future benefits of this vaccine will accrue as younger generations vaccinated in childhood reach the ages where liver cancer becomes common. The WHO recommends its inclusion in routine infant immunization programs and, by the end of 2016, 186 countries had introduced the HBV vaccine into their national immunization schedules with many countries achieving greater than 80% coverage for the full recommended dose.[12] The vaccine has dramatically reduced the prevalence of HBV infection and the incidence of HCC at younger ages in high-risk countries in East Asia, where mass vaccination was first introduced.[13] However, currently, there is no vaccine available to prevent HCV infection. Although there have been substantive declines in HCV transmission in highly resourced countries, the continued use of contaminated needles and unsafe transfusions contribute to the spread of infection in several low-income countries.[14]

Colorectal cancer Over 1.8 million new colorectal cancer cases and 881,000 deaths are estimated to occur in 2018, accounting for about 1 in 10 cancer cases and deaths. Overall, colorectal cancer ranks third in terms of incidence but second in terms of mortality. Colorectal cancer incidence rates are about 3-fold higher in transitioned versus transitioning countries; however, with average case fatality higher in lower HDI settings, there is less variation in the mortality rates. Colorectal cancer incidence rates vary widely, with 8-fold and 6-fold variations in colon and rectal cancer, respectively, by world region; the disease can be considered a marker of socioeconomic development and, in countries undergoing major development transition, incidence rates tend to rise uniformly with increasing HDI.[15,16] Assessing incidence and mortality trends the types of cells that most commonly give rise to colorectal cancers is the adenocarcinoma which is cancer of the cells that line the inside tissue of the colon

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and rectum.[17] Treatment efficiency of colorectal and liver cancer is low, therefore colorectal and liver cancer prevention and control is extremely important. Recent developments in the treatment of HBV and HCV suggest that large numbers of liver cancer cases could be avoided, although the costs are prohibitive at present.[10] *Ruellia tuberosa* L. (Acanthaceae), is a tropical plant which is widely distributed in Southeast Asia. In folk medicine, it has been used as diuretic, antidiabetic, antipyretic, analgesic, antihypertensive and antidotal agent. It has different names such as Minnie Root, Fever Root, Snapdragon Root and Sheep Potato. In Sabah, Malaysia, the plant is called by the local as "Cracker Plant" as the seeds burst when comes in contact with water. The plants can be easily found in the shady and moisture place such as side drain. Recently, it has been incorporated as a component in an herbal drink in Taiwan. However, very few chemical constituents and pharmacological activities have been reported on this species. The whole plant has been shown to possess antimicrobial activity and the stem has also been shown to possess antioxidant activity.[18,19]

The anticancer activity of *Ruellia tuberosa* leaf extract (RTLE) using hepatocellular carcinoma (Huh-7), colorectal cancer (SW480) and its effect on normal human kidney (HEK293T) was studied. The cytotoxic activities of RTLE were supported by the observations in MTT assays. RTLE showed the anti-proliferative activities of metabolically active Huh-7 & SW480 cells in a concentration and time dependent manner. We also studied the toxic effect of RTLE on HEK293T cells. In MTT assay of RTLE treated HEK293T cells insignificant inhibition took place in a non-concentration and time dependent manner. Anti-cancer activity of RTLE was investigated by different morphological studies like fluorescence microscopic, confocal microscopic in hepatocellular carcinoma cells. The process of apoptosis is characterized by several morphological changes such as cell shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies. Fluorescence microscopic images clearly showed nuclear disintegration of RTLE treated hepatocellular carcinoma cells compared with that of the untreated control cells when stained with acridine orange and ethidium bromide. The untreated control cells showed bright green fluorescence. On the contrary RTLE treated cells showed more intense orange-red fluorescence and reduced green fluorescence. The observations indicated that the treatment of Huh-7 cells with RTLE shows apoptosis. Apoptosis activity of RTLE was further evidenced from the confocal microscopic images of the treated hepatocellular carcinoma cells when compared with that of the untreated control cells. After RTLE treatment, Huh-7 cells showed several signs of apoptosis like chromatin condensation, nuclear fragmentation and formation of apoptotic bodies whereas the untreated control cells were with intact nuclei.

Further evidence in support of the anti-cancer activity of RTLE was obtained from the gel patterns of agarose gel electrophoresis. RTLE treated both the cells showed degraded DNA bands in the form of ladders, a typical indication of apoptosis, whereas the untreated control cells showed intact DNA bands when observed in UV transilluminator. The mitochondrial membrane potential assay by JC-1dye showed increase in mitochondrial membrane potential in both Huh-7 & SW480 cells but did not increase in case of Hek293T cells. Experiments also showed increased number of cells in the late apoptotic stage after treatment of Huh-7 cells& SW480cells with RTLE which implies the fact that apoptosis was triggered by the treatment with RTLE in Huh-7 cells& SW480cells but not in case of Hek293T cells. Cell cycle analysis revealed that treatment of Huh-7& SW480cells with RTLE arrested the Huh-7 & SW480cells cell populations in the sub G_0/G1 phase of cell cycle. However, RTLE did not change populations of Hek293T cells in the sub G_0/G1 phase of cell cycle.

CONCLUSION

It has been concluded that RTLE possess anti-proliferative and cytotoxic effect against hepatocellular carcinoma cell (Huh-7) and Colorectal cancer (SW480) cells but it shows insignificant inhibition and non- apoptotic activity against normal human embryonic kidney cells (HEK293T). Therefore, we can conclude that RTLE specifically shows activity in cancer cells. The plant *Ruellia tuberosa* possesses lots of medicinal value. It can be considered as a potent anti-cancer agent for treatment of hepatocellular carcinoma and Colorectal cancer with less side effects. Further mechanistic study should be done and identify the active compounds which are responsible for anticancer activity of *Ruellia tuberosa* on both the adenocarcinoma.

Acknowledgement: The authors of this paper are very much thankful to Council of Scientific and Industrial Research, Indian Institute of Chemical Biology (CSIR-IICB), Kolkata for providing the platform to perform the research work.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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