

Research Article



Mechanistic Evaluation of White Tea Extract on Human Lymphoid and Myeloid Leukemia Cell Lines

Antara Haldar, Shila Elizabeth Besra*

Central Instrumentation Facility and Cancer Biology & Inflammatory Disorder Division, CSIR-Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata-700032, West Bengal, India.

*Corresponding author's E-mail: shilabesra@iicb.res.in, shilabesra@gmail.com

Received: 10-09-2022; Revised: 19-11-2022; Accepted: 26-11-2022; Published on: 15-12-2022.

ABSTRACT

Health benefits have been attributed to tea consumption since the beginning of its history. White tea contains a high amount of antioxidants, as well as polyphenols, flavonoids, and tannins which keep well-nourished and protect health. We evaluated the antineoplastic potential of white tea extract (WTE) on lymphoblastic and myeloblastic leukemia cell lines. WTE significantly inhibited the cell viability in a time dependent manner in all the lymphoid and myeloid leukemia cells. After the treatment with WTE, leukemia cells showed various signs of apoptosis like nuclear fragmentation, formation of apoptotic bodies, chromatin condensation and degraded DNA bands in cancer cells. The presence of apoptotic cells in the early and late stages was confirmed by the flow cytometric analysis. The cell cycle phase arrest was observed in the G0/G1 phase in all the leukemia cells. A significant change in the mitochondrial membrane potential was observed in the WTE treated myeloid lymphoma and the multidrug resistant leukemia cells with MMP shift assay. Activation of Caspase 9 and 3 and reduction in Caspase 8 confirms the apoptosis was mediated through intrinsic pathway activation. These finding suggest that the White Tea extract (WTE) possesses anti-leukemic effects via apoptosis and not necrosis without toxicity.

Keywords: White Tea, Leukemia, Lymphoid, Myeloid, Apoptosis.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2022.v77i02.012



DOI link: <http://dx.doi.org/10.47583/ijpsrr.2022.v77i02.012>

INTRODUCTION

Tea is the second most consumed drink after water and is consumed by people of all ages. Tea is a plant based beverage and is obtained from leaves of *Camellia sinensis* plant. Leukemia refers to abnormal and uncontrolled proliferation of blood cells in the bone marrow due to genetic and epigenetic changes. In this disorder hematopoietic stem cell starts producing more immature WBC and replaced with normal WBC, resulting in the immune-compromised condition. Leukemia is one of the most common cancers in people over 55 years in the developed countries. It is estimated that 61,780 are the new cases of leukemia and 22,840 is leading to death. The latest figures show that the 5-year survival rate for all subtypes of leukemia is 62.7% (US, NCI 2019). Only chemotherapy is an effective method for the treatment of malignant leukemia. Nowadays, several therapeutic approaches have been taken to beat the complexities of various cancers. The greatest disadvantage in the presently accessible potent artificial anti-cancer drugs lies in their toxicity, many side effects, and reappearance of symptoms after discontinuation¹. Cytarabine (cytosine arabinoside,

ARA-C) has been used for the remedy of acute myeloid leukemia (AML) for nearly 40 years.² Drug resistance one of the main obstacles of chemotherapy. In the case of ARA-C resistance can occur by several different mechanisms.³ ARA-C is a prodrug that's triggered by phosphorylation by deoxycytidine (CdR) kinase. Because the human CdR kinase gene is placed on chromosome 4, there are two copies of the gene in the cell.⁴ Complete drug resistance due to a deficiency in CdR kinase is unlikely because it lacks the gene inactivation of both alleles. Another mechanism of drug resistance to ARA-C is due to an increase in the intracellular level of dCTP in leukemic cells.⁵ To avoid this complication, it is necessary to use natural methods for the treatment of leukemia. Tea is one of the most popular drinks due to its pleasant taste and perceived health effects. Although health benefits have been attributed to tea consumption since the beginning of its history, scientific investigation of this beverage and its constituents has been under way for about 30 years.^{6,7} White tea contains a high amount of antioxidants, as well as polyphenols, flavonoids, and tannins. These nutritional benefits have a positive effect on our health and wellbeing. White tea has been shown to protect the body against certain diseases and reduce the risk of cancer and cardiovascular disorders.⁸ It also provides natural antibacterial properties, helps with weight loss, and can lower bad cholesterol. Some studies suggest that white tea increase the antioxidant effects of bodily organs as well as increase the plasma.⁹ White tea extract (WTE) also showed high anti-proliferative activity against HT-29 cells, without being toxic to normal fibroblasts. The extract inhibited HT-29 colon cancer cells by the death receptor and



mitochondrial apoptotic pathways as demonstrated by increased expression levels of caspases-3/7, -8 and -9.¹⁰ Therefore, we evaluated the anti-leukemic activity of bark extract of White Tea extract (WTE) against MOLT-4 (human T lymphoblast; acute lymphoblastic leukemia), U937 (human leukemic monocytes lymphoma cell line), K562 (human myelogenous leukemia cell line) cell line.

MATERIALS AND METHODS

Chemicals

The following chemicals were used: RPMI 1640 medium (Gibco, USA), Fetal bovine serum (FBS), HEPES, L-glutamine, Penicillin- Streptomycin (Bio-west, Germany), Gentamycin (Nicholas, India), Ara-C (Arabinofuranosyl Cytidine), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide], Ethidium bromide and Acridine orange, Annexin V- FITC apoptosis detection kit, RNase, Propidium iodide, were purchased from Sigma (St. Louis, MO, USA), Proteinase k (SRL), Caspase-8, Caspase-9, Caspase-3 (Bio-Vision), DMSO (dimethylsulphoxide), Chloroform, isoamyl alcohol, Methanol (Merk), and all other chemicals and reagents were of analytical grade and procured locally.

Cell culture

Three human leukemic cell lines MOLT-4, U937, K562 & RAW264.7 were purchased from the National Facility for Animal Tissue and Cell Culture, Pune, India. The cells were maintained in RPMI 1640 medium (Gibco, USA), supplemented with 10% heat inactivated FCS, 100U/ml Penicillin (Biowest, Germany), and 100mg/ml Streptomycin (Biowest, Germany). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂ in air. In all the experiments untreated leukemic cells were termed as control group.

Collection and Preparation of Test Sample

Most tea aficionados know that all tea comes from the same source: The *Camilla Sinensis* tea bush. White Tea comes from the fine silvery-white hairs on the unopened buds of the tea plant. White tea usually requires only minimal processing: the immature leaves from the tea bush are plucked fresh and the tea leaves are withered by air-drying, solar-drying, or mechanical drying. White Tea was Purchased from Amazon. in (Silver Leaf Tea Pvt. Ltd.) Batch Code: 0917, Mfg. Date: 09/2017. White Tea extract (WTE) was prepared by dissolving 0.5g of WTE in 25 ml of boiling water at boiling temperature (100°C) and brewed for 5 minutes according to Fatimah *et al*, 2014.⁸ The samples were then filtered using a Millipore filter membrane (0.22µm) and desired dose of tea was used for all different studies.

Detection of Cytotoxicity by MTT assay

Molt-4, U937, K562 & RAW264.7 cells (1x10⁵) were separately seeded in 96-well sterile plates for 24, 48 and 72 hrs. All the treated cells were grown in humidified atmosphere containing 5% CO₂ in an incubator at 37°C and the untreated cells were considered as control. After

desired 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. The MTT assay is a colorimetric assay for assessing the metabolic activity of the cells or cell viability of NADPH dependent cellular oxidoreductase 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 coloured formazan. The intensity of the colour was measured at 492nm by micro-plate manager (Reader type: Model 680XR Bio-Rad Laboratories Inc.). The IC₅₀ values were determined for the all the carcinoma cells.

Fluorescence Microscopy

Molt-4, U937 and K562 (1x10⁶) were treated with different IC₅₀ doses of WTE (corresponding to each cell line) for 24 hrs and observed using a fluorescence microscope for determining morphological changes. The untreated control cells and WTE treated cells were harvested separately (centrifuged at 1000 rpm for 5 min), the pellets were washed twice with PBS and then stained with 100µg/ml of acridine orange (Sigma, USA) and 100µg/ml of ethidium bromide (Sigma, USA) in a ratio of 1:1. The cells were then immediately mounted on slides and observed under a fluorescence microscope for the morphological determination of the cells undergoing apoptosis.

Confocal Microscopy

Molt-4, U937 and K562 cells (1x10⁶) were treated with different IC₅₀ doses of WTE for 24 hrs. After 24hrs the untreated control cells and WTE treated cells were harvested and washed with ice cold PBS. The cells were then stained with 10µg/ml of Propidium iodide (Sigma) for 5 min. After mounting on slides the cells were observed to determine the differences in nuclear morphology between the untreated and WTE treated leukemic cells under confocal laser scanning microscope (Leica TCS-SP2 system, Leica Microsystem, Heidelberg, Germany) installed with an inverted microscope (LeicaDM-7RB).⁸ Images for Propidium iodide was acquired from UV laser line using 450 nm band pass filter for UV for images.

Agarose gel electrophoresis study

Molt-4, U937 & K562 cells were treated with IC₅₀ dose of WTE for 14 hrs then cells were resuspended in 500µl of lysis buffer (50 mMTris- Hcl, pH -8.0, 10 mM EDTA, 0.5% SDS), 100µg/ml of proteinase K was added and incubation was done at 50°C for 1 h and 37°C overnight respectively. 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% ethanol, dried and evaporated at room temperature and dissolved in TE 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 transilluminator¹⁰.



Mitochondrial membrane potential ($\Delta\psi_m$) assay

U937 and K562 (1×10^6) cells were treated with WTE with desired dose and untreated as control for 24 hours to assay the mitochondrial membrane potential activity of cell in a flow cytometer. Cells were washed with PBS, pelleted down and eventually stained with JC-1 stain. The sample were incubated at 37°C for 15 min. Change in the mitochondrial membrane potential was determined by FACS (Becton Dickinson FACS Fortessa 4 leaser cytometer), Fluorescence detector equipped with 520 nm argon laser light source and 623 nm band pass filter (linear scale) with the help of BD FACS Diva software (Becton Dickinson).

Apoptosis Assay

In order to investigate the type of cell death induced by WTE, flow Cytometric analysis was done by performing dot plot assay. The U937 and K562 cells (1×10^6) were treated with individual IC_{50} dose (18 hrs) of WTE for 18 hrs. The cells were centrifuged at 2000 rpm for 8 min at 4°C and pelleted down. Then washed with Annexin-V- FITC binding buffer provided in apoptosis kit (Sigma). 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 LSR Fortessa 4 laser Cytometry. Flow-Cytometry 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 BD FACS Diva software program¹¹.

Cell Cycle Arrest Study

To assay the stage of cell cycle arrest in a flow cytometry Molt-4, U937 and K562 (1×10^6) cells were treated with WTE (IC_{50} dose) for 18 hrs. Cells were washed with PBS, fixed with cold methanol. 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 for 30 min at 37 °C and stained with Propidium iodide and kept in dark for 15 min. Cell cycle phase distribution of nuclear DNA was determined on FACS (Becton Dickinson FACS Fortessa 4 laser cytometer), fluorescence detector equipped with 488 nm argon laser light source and 623 nm band pass filter (linear scale) using BD FACS Diva software (Becton Dickinson).¹¹

Caspase-8 Assay

The assay was performed using a Caspase-8, Apoptosis Detection, Caspase-8/FLICE Colorimetric Bio-Assay Kit (Bio Vision), Cat.K113-100 according to the manufacturer's protocol. U937 cells (1×10^7) were treated with IC_{50} dose for 24 h. The cells were pelleted down and resuspended in 50 μ l of cell lysis buffer (supplied with the kit) and incubated on ice for 10 min. After centrifuging at 10,000 x

g 1 minute, the supernatants (cytosolic extract) were transferred to fresh tubes and kept on ice and the caspase-8 assay was performed according to the supplied kit protocol. 50 μ l of 2X reaction buffer (containing 1M DTT) was added to each sample. 5 μ l of IETD-pNA substrate (4 mM) (200 μ M final concentration) was added and incubation was done at 37 °C for 1-2 h. Absorbance was read at 405 nm and calculations were thereby done.¹²

Caspase-9 Assay

The assay was performed using a Caspase-9, Apoptosis Detection, Colorimetric Bioassay Kit (R&D Systems), Catalog No.BF10100 according to the manufacturer's protocol. U937 cells (1×10^7) were treated with IC_{50} dose for 24 h. The cells were pelleted down and resuspended in 50 μ l of cell lysis buffer (supplied with the kit) and incubated on ice for 10 min. After centrifuging at 10,000 x g for 1 minute, the supernatants (cytosolic extract) were transferred to fresh tubes and kept on ice and the caspase-9 assay was performed according to the supplied kit protocol. 50 μ l of 2X reaction buffer (containing 1M DTT) was added to each sample. 5 μ l of LEHD-pNA substrate (4 mM) (200 μ M final concentration) was added and incubation was done at 37 °C for 1-2 h. Absorbance was read at 405 nm and calculations were thereby done.¹²

Caspase-3 Assay

The assay was performed using a Caspase-3, Apoptosis Detection, Colorimetric Bio-Assay Kit (R&D Systems), Catalog No.BF3100 according to the manufacturer's protocol. U937 cells (1×10^7) were treated with IC_{50} dose for 24 h. The cells were pelleted down and resuspended in 50 μ l of cell lysis buffer (supplied with the kit) and incubated on ice for 10 min. After centrifuging at 10,000 x g for 1 minute, the supernatants (cytosolic extract) were transferred to fresh tubes and kept on ice and the caspase-3 assay was performed according to the supplied kit protocol. 50 μ l of 2X reaction buffer (containing 1M DTT) was added to each sample. 5 μ l of DEVD-pNA substrate (4 mM) (200 μ M final concentration) was added and incubation was done at 37 °C for 1-2 h. Absorbance was read at 405 nm and calculations were thereby done.¹²

Statistical Analysis

Statistical analysis was done by Student's *t*-test. $P < 0.05$ was considered as significant.

The percentage cell inhibition was calculated by the following formula: - %Cell inhibition= $100 \times (O.D \text{ of control} - O.D \text{ of treated}) / O.D \text{ of control}$. O. D= Optical Density.

The percentage cell viability was calculated by the formula: - Viable cells (%) = $(\text{Total number of viable cells per ml} / \text{Total number of cells per ml}) \times 100$.



RESULTS AND DISCUSSION

Detection of Cytotoxicity by MTT assay

MTT assay of WTE showed very convincing cytotoxic effect on all the leukemic cell lines Molt-4, U937, K562, & RAW264.7. The OD value is progressively decreases with

time (24, 48 & 72 hrs.) and the concentration dependent manner of WTE (25, 50, 100, and 200 µg). The IC₅₀ value of WTE treatment for 24hrs were calculated to be **153.8 µg/mL** for U937, **29.7 µg/mL** for K562 and **58.85 µg/mL** for Molt-4 cells respectively.

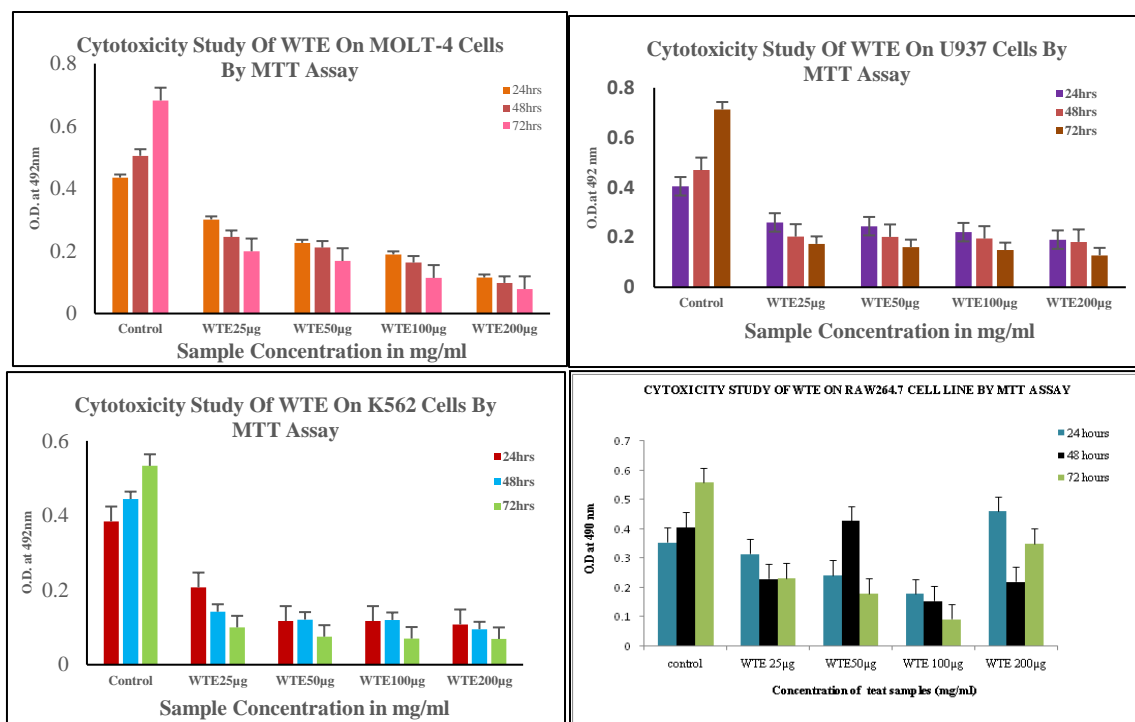


Figure 1: Histogram showing the significant decrease in OD value of Molt-4, U937, K562 & RAW264.7 cells after treating with WTE than control. WTE treated cells showing progressively increase in % of inhibition with time and concentration. The IC₅₀ value of treated cells for 24hrs was calculated to be 58.85 µg/mL for Molt-4, 153.8 µg/mL for U937 and 29.7µg/mL for K562 cells respectively.

Morphological study by Fluorescence Microscope

Fluorescence microscopic images clearly showed the membrane disintegration on Molt-4, U937 and K562 cells treated with WTE compared to that of untreated (control) cells. Molt-4, U937 and K562 was treated with WTE with IC₅₀ value for the stained with ethidium bromide and acridine orange (colour-red or orange), investigate the presence of apoptotic cells (early and late) as compared to the untreated control cells stained with only acridine orange (colour-green). In this study nuclear changes were observed including chromatin condensation and apoptotic body formation that indicate the occurrence of apoptosis. WTE induced apoptotic changes in both the leukemic cells. After 24 hrs of treatment showing chromatin disintegration and formation of apoptotic bodies whereas the untreated cells have with intact nuclei.

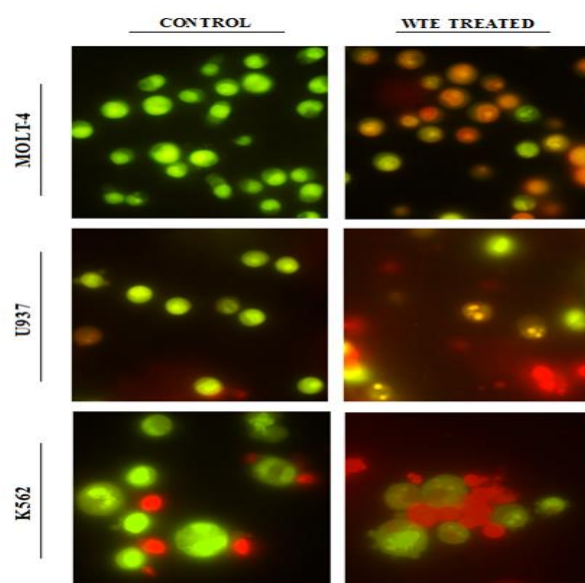


Figure 2: Fluorescence microscopic images of control vs treated cell line of Molt-4, U937 and K562 with IC₅₀ dosage. The Untreated (control) cells shows intact morphology and gives bright green fluorescence whereas the treated cells show distorted morphology due to fragmented nuclei which ensure the apoptosis.

Morphological study by confocal Microscope

WTE shows a potent anti-leukemic effect after 24 hrs of treatment. Which showed the disintegration of nuclei and formation of apoptotic body and the control was remain same.

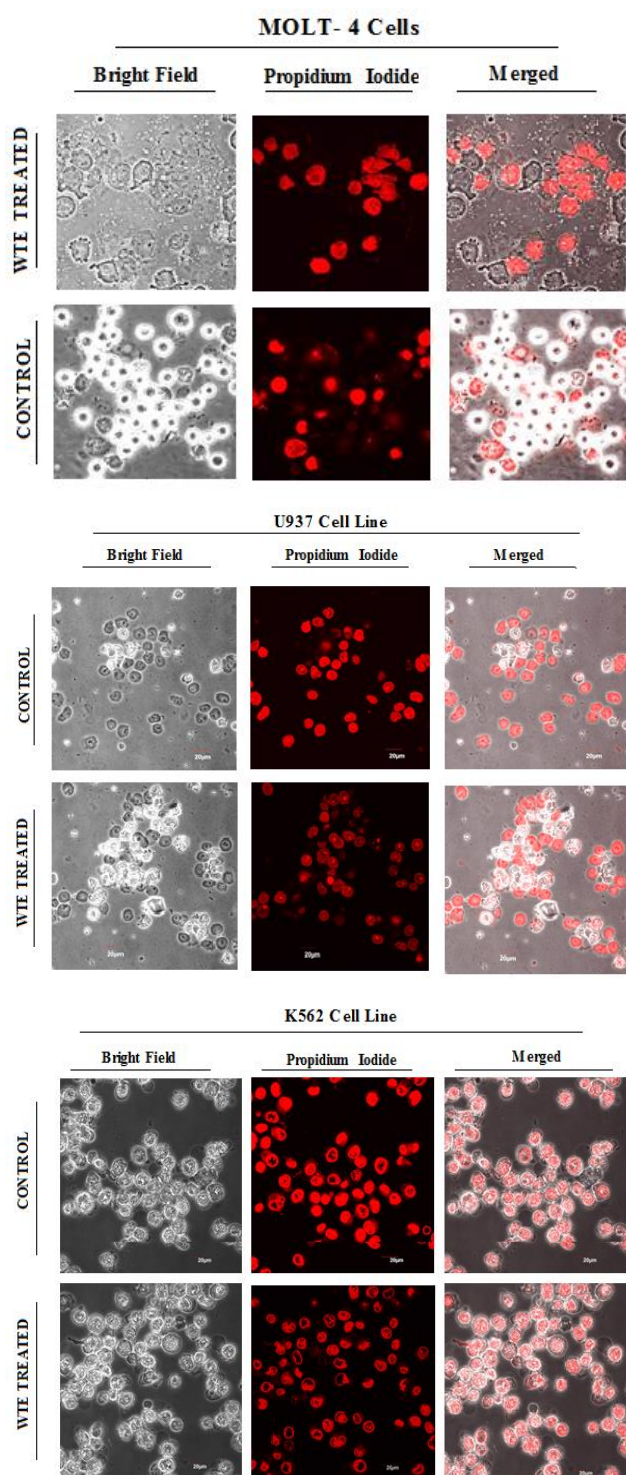


Figure 3: Confocal microscopic images of control vs treated cell line of MOLT -4, U937 and K562 with IC₅₀ dosage. The Untreated (control) cell shows intact morphology whereas the treated cells show distorted morphology due to fragmented nuclei and formation of apoptotic body take place. The cells were stained with PI.

Agarose gel electrophoresis study

The DNA samples were isolated from both treated as well as untreated cells. The untreated (control) cells show intact DNA (lack cleavage) of Molt-4, U937 and K562. Whereas treated cells showed fragmented DNA of Molt-4, U937 and K562 on agarose gel. This simply indicates that WTE showing apoptotic activity in Molt-4, U937 and K562 Cells.

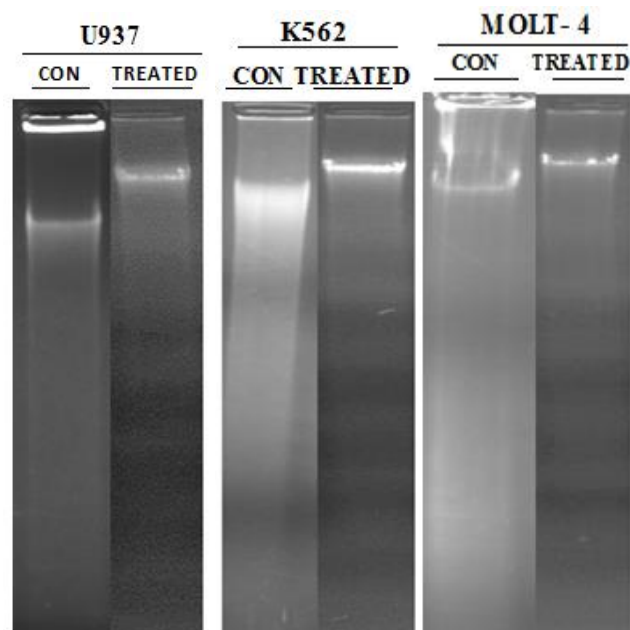


Figure 4: Agarose gel images of control vs treated cell line of MOLT-4, U937 and K562 with IC₅₀ dosage. Lane 1 represents control of MOLT-4, U937and K562 respectively. Lane 2 represents WTE with IC₅₀ dosage on MOLT-4, U937& K562 cell lines respectively.

Detection of mitochondrial membrane potential ($\Delta\psi_m$) assay

Disruption of mitochondrial membrane potential is a very important parameter for the induction of apoptosis. The U937 and K562 cell lines when treated with desired dose of WTE, showed a loss of Mitochondrial Membrane Potential ($\Delta\psi_m$). The JC-1 stain cannot persist 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 persist. Depolarization in mitochondrial membrane potential was observed by staining untreated and treated cell by JC-1 dye. It led to shift from red to green fluorescence leading to the release of Cytochrome C. A efficient transmembrane shift of 9.6% to 81.1% and 9.9% to 50.6 % were observed when U937 and K562 cells were treated with the IC₅₀ value of WTE for 24 hours respectively.

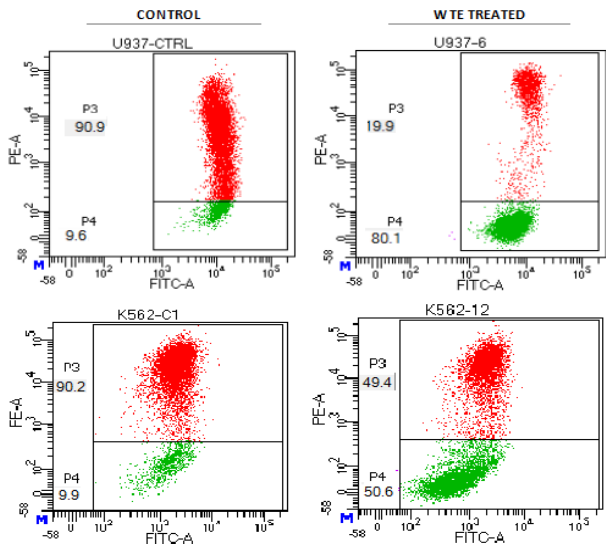


Figure 5: Flow cytometric analysis of mitochondrial membrane potential ($\Delta\psi_m$) on U937 and K562 cell line in both control and WTE treated cells respectively after 24 hrs of treatment. A significant shift red to green fluorescence was observed in U937 and K562 cells.

Detection of Apoptosis by Flow Cytometry

In the flow cytometric analysis, double labelling 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 and

extreme upper right (UR) & upper left (UL) quadrant (annexin V-/PI+) is considered as necrotic cell population. Flow cytometric data analysis revealed that after 24 h of treatment with desired dose of WTE for quantification of apoptosis was observed for U937 0.1% against 37.2% and for K562 2.5% against 61.2%, cells were in lower right quadrant which implies early apoptotic stage thereby, showing apoptotic inducing property of WTE on U937 and K562.

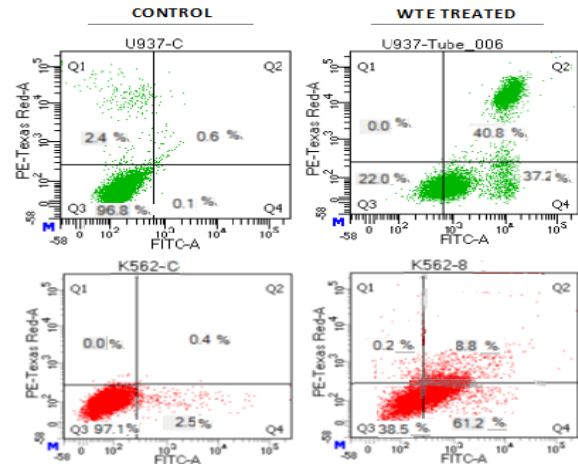


Figure 6: Flow cytometric analysis of untreated control and WTE treated of U937 and K562 cells stained with Annexin V FITC and propidium iodide. Dual parameter dot plot of FITC fluorescence (x-axis) vs PI-fluorescence (y-axis) shows logarithmic intensity.

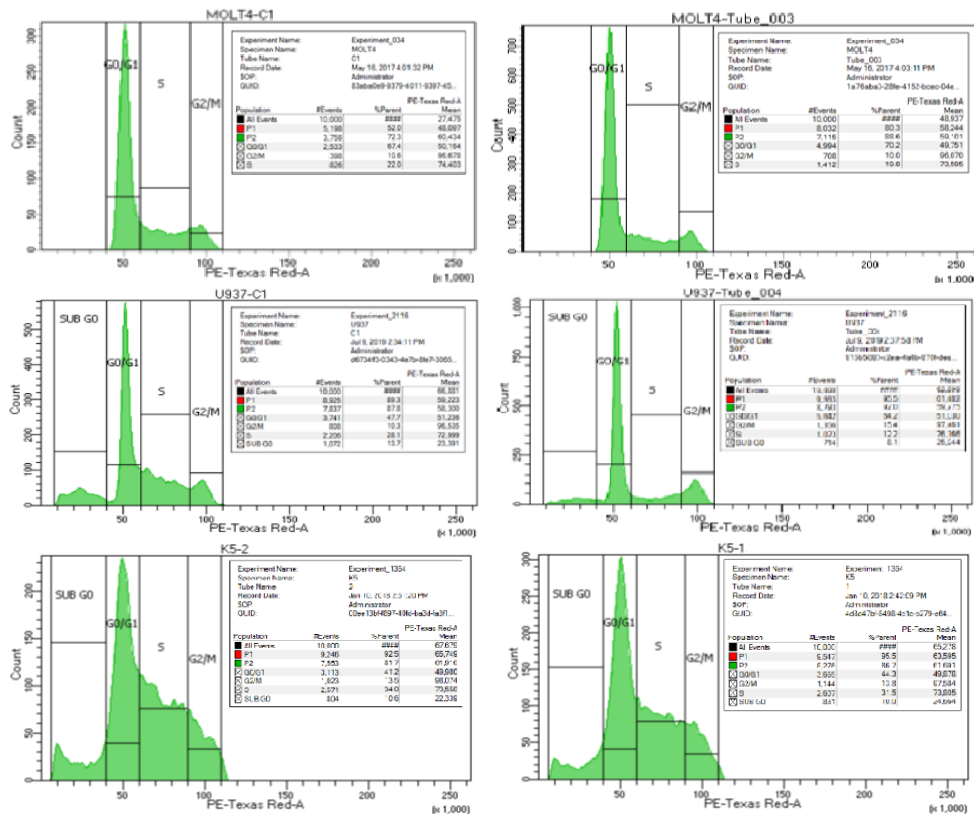


Figure 7: Flow cytometric evaluation of cell cycle phase distribution in controls and treated WTE at IC50 dosage on MOLT-4, U937 & K562 cell after 24 hrs of treatment. Histograms represent various contents of DNA with actual number of cells (x-axis denotes fluorescence intensity of PE- Texas red and y-axis denotes count).



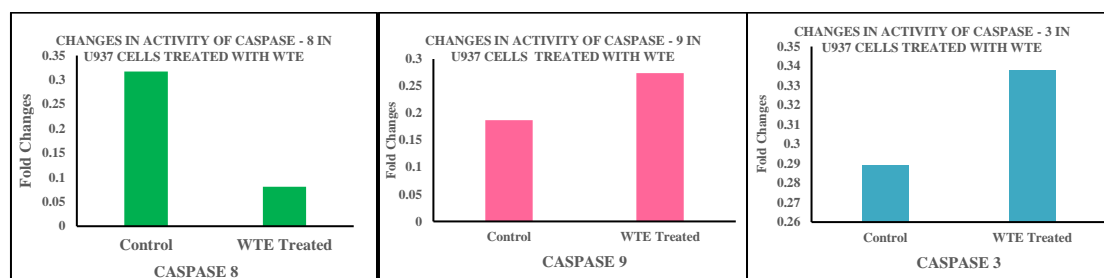


Figure 8: Effect of WTE treatment on Caspase activity in U937 cells after 24hrs at IC₅₀ dose with respect to control. The activity of caspase-8 is decreases which suggest apoptosis induced by WTE treatment might not be mediated through the extrinsic pathway. Whereas in caspase-3 and 9 activities was increases which show WTE treatment follow the intrinsic pathway.

Study of Cell Cycle Arrest by Flow Cytometry

Flow cytometric evaluation showed that after 24hrs treatment of MOLT-4 cell line DNA content increased in G1 phase (70.2% against 57.4%). In U937 cell line DNA content increased in G1 phase (64.2% against 47.7%). In case of K562 cell line DNA content increased in G1 phase (64.3% against 41.2%). This observation demonstrate that WTE significantly inhibited the growth of K562 and U937 cell line by arresting the cell populations in the sub-G0/G1 phase of the cell cycle.

Caspase-8

Caspases are a family of proteases that play a key role in cellular process in apoptosis. To investigate whether treatment with WTE induced apoptosis via extrinsic pathway, caspase-8 assays were performed in U937 cells. The experiments revealed insignificant change in the caspase-8 activity in the extract of WTE treated with IC₅₀ dose, as compared with that of the untreated control U937 cells. (Fig.9), supporting the fact that apoptosis induced by WTE treatment might not be mediated through the extrinsic pathway.

Caspase -9 assay

To investigate whether treatment with WTE induced apoptosis via intrinsic pathway, caspase-9 assays were performed in U937 cells. It shows significant change caspase-9 activity that suggests WTE induce might be via intrinsic pathway.

Caspase-3 assay

To investigate whether treatment with WTE induced apoptosis via intrinsic pathway, caspase-3 assays were performed in U937 cells. It shows significant change caspase-3 activity that suggests WTE induce might be via intrinsic pathway.

DISCUSSION

Cancer is the largest cause of death worldwide, with approximately 10 million deaths predicted in 2020, accounting for nearly one in every six deaths.¹³ Lifestyle is responsible for up to 95% of all cancers, which can take 20–30 years to develop. Cancers of the blood cells are referred to as leukaemia. The kind of leukaemia is determined by the type of cancerous blood cell and its rate of growth.¹⁴ Many

people with leukaemia that grows slowly don't show any signs or symptoms. The course of treatment varies widely. Treatment may include monitoring for slow-growing leukemia. Chemotherapy is followed by radiation and a stem-cell transplant and used to treat aggressive leukemia.¹⁵ Natural-derived substances are gaining popularity among scientists and specialists since they have less severe side effects than conventional treatments like chemotherapy. Tea is an anti-oxidant, anti-inflammatory, antibacterial, antidiarrheal and analgesic herb that has been consumed for centuries. But white tea has been not done so far. Therefore, we investigated the white tea extract on against lymphoid (Molt4) and myeloid Leukemia (U937 & K562) cell lines. MTT assay results indicated antiproliferative and cytotoxic properties. The extract reduced cell growth and metabolic activities of the cell line in a time and dose dependent way. According to this study, WTE has an effect on leukemic cells. The effect of WTE on cell morphology was investigated further using a fluorescence microscope. Several morphological signs of apoptosis can be observed, including cell shrinkage, fragmentation into membrane-bound apoptotic cell, and rapid phagocytosis by nearby cells. After staining with acridine orange and ethidium bromide for fluorescence microscopy, the fluorescence microscopic images of the cells clearly showed nuclear degeneration of WTE treated U937 cells, compared to untreated control cells. The untreated control cells emitted bright green colour and had an intact cell membrane, as it prevents to enter ethidium bromide in case of fluorescence microscope. MEMPL treatment causes apoptosis in the cells according to this research. Cell cycle phase distribution was also studied by flow cytometrically at IC₅₀ doses in control and MEMPL-treated U937 cells. Mitochondria of the normal cells can take JC-1 dye and produce red pigment. But in apoptotic cells, the mitochondrial membrane potential is disrupted, and the JC-1 dye is unable to enter within the mitochondria. The release of cytochrome C was induced by a transmembrane shift from red to green fluorescence caused by this depolarization. After staining with JC-1 dye, U937 cells treated with MEMPL showed a significant shift in transmembrane potential from red to green fluorescence. Healthy cells with predominately red fluorescence can be separated from apoptotic cells with principally green fluorescence. From the result, the findings suggest that a

shift in transmembrane potential induces apoptosis. Another study compares the quantitative impact of early vs. late apoptosis in cells. It was also supported by dot plot assay which used dual staining with annexin V FITC and propidium iodide. Phosphatidylserine (PS) Externalization is a hallmark of apoptosis, and various indicators for the study have been discovered. The binding of Annexin-V, which is labelled with FITC, is permitted by the translocation of PS molecules to the cell's external surface. In dot plot assay, it was possible to identify live, early apoptotic, and late apoptotic cells using a dual staining technique with annexin V FITC and propidium iodide. The study showed the increase in the number of early and by the treatment with WTE of IC₅₀ dose in U937 cells. One of the main causes of cancer progression is the deregulation of checkpoints. It eliminates cell irregularities and assures that cells follow the correct sequence of events of cells through the cell cycle's different phases. From the study of cell cycle arrest by flow cytometry, it clearly proved that arresting cell populations in the sub-G₀/G₁ phases of the cell cycle slowed the growth of the U937 cell line.

CONCLUSION

The present investigations confirmed that White Tea extract (WTE) shows significant effect on leukemic cells (monocytes lymphoma, myelogenous leukemia and, acute promyelocytic leukemia cells) by triggering programmed cell death but it shows insignificant and non- apoptotic activity against normal murine macrophage cells (RAW264.7). Therefore, WTE efficiently shows the cytotoxic effect on human leukemia cell line via Caspase activation. So, from these studies it can be considered as a potent anti-leukemic agent for treatment of leukemia with minimum side effects. Further, more studies should be done and identify the active compound as well as to identify the signal transduction pathways in White Tea for the treatment of various types of chronic diseases that will be beneficial for the human society.

Acknowledgement: The authors are very grateful to Indian Institute of Chemical Biology, Kolkata for providing facilities to perform the work. The authors are also thankful to CSIR for providing the necessary funding for the project.

REFERENCES

- Lowenberg B, Downing JR, Burnett A., Acute myeloid leukemia, *New England Journal of Medicine*, 1999;341:1051-62.
- Momparler RL, Onetto-Pothier N. In: Kassel D, ed., *Resistance to Antineoplastic Drugs*, Boca Raton: CRC Press, 1998; 353–367.
- Momparler RL, Chu MY, Fischer GA., Studies on a new mechanism of resistance of L5178Y murine leukemia cells to cytosine arabinoside, *Biochimica et Biophysica Acta (BBA)-Nucleic Acids and Protein Synthesis*, 1968;161:481-93.
- Flasshove M, Strumberg D, Ayscue L, Mitchell BS, Tirier C, Heit W, Seeber S, Schütte J., Structural analysis of the deoxycytidine kinase gene in patients with acute myeloid leukemia and resistance to cytosine arabinoside, *Leukemia*, 1994, 8, 780.
- McKay D.L, Blumberg J.B. The role of tea in human health: An update. *J Am Coll Nutr*. 2002;21(1):1–13.
- Gardner E.J, Ruxton C.H, Leeds A.R. Black tea-helpful or harmful? A review of the evidence. *Eur J Clin Nutr*. 2007;61(1):3–18.
- Dias TR, et al. White Tea (*Camellia sinensis* (L.)): Antioxidant Properties and Beneficial Health Effects. *Int J Food Sci Nutr Diet*. 2013;2(2):19-26. doi:10.19070/2326-3350-130005
- Pastoriza S, Mesías M, Cabrera C, Rufián-henares JA. Healthy properties of green and white teas: an update. *Food Funct*. 2017;8(8):2650-2662. doi:10.1039/C7FO00611J
- Naghma Khan and Hasan Mukhtar, Tea polyphenols for health promotion, *LifeSci*. 2007 Jul 26;81(7):519-533.
- Fatemeh H, Kanthimathi MS. White tea (*Camellia sinensis*) inhibits proliferation of the colon cancer cell line, HT-29, activates caspases and protects DNA of normal cells against oxidative damage. *Food Chemistry* 2014; 169: 401-410.
- Queiroz AC, Contrera FA, Venturieri GC., The effect of toxic nectar and pollen from *Spathodea campanulata* on the worker survival of *Melipona fasciculata* Smith and *Melipona seminigra* Friese, two Amazonian stingless bees, *Sociobiology*, 2014;61:536-40.
- Shrawan Kumar, Rahul Laxhman Gajbhiye, Shila Elizabeth Besra, Apoptosis Inducing Activity of Bark Extract of *Spathodea campanulata* on Human Leukemia Cell Lines U937, K562 & HL60 Cell Lines via *Caspase Cascade*, *Int. J. Pharm. Sci. Rev. Res.*, 2020; 64(1):114-122
- Wagh AS, Butle SR., Plant profile, Phytochemistry and pharmacology of *Spathodea campanulata* p. Beauvais (African tulip tree): a review, *IJPPS*, 2018;10:0975-1491.
- Herrmann M, Lorenz HM, Voll RE, Grünke M, Woith W, Kalden JR., A rapid and simple method for the isolation of apoptotic DNA fragments, *Nucleic acids research*, 1994;22:5506.
- Jacobson MD, Weil M, Raff MC., Programmed cell death in animal development, *Cell*, 1997;88:347-54.

Source of Support: CSIR- Indian Institute of Chemical Biology, Kolkata was providing the financial support for this work.

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

For any question relates to this article, please reach us at: globalresearchonline@rediffmail.com
New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

