

Research Article



Apoptosis Inducing Activity of *Diospyros melanoxylon* Bark Extract on Molt-3 Cell Line via Activation of Mitochondrial Membrane Caspase Cascade and Cell Cycle Arrest with its Antioxidant Potential

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ABSTRACT

The present work aimed to investigate the apoptosis activity of *Diospyros melanoxylon* bark extract (DMBE) on Molt-3 human acute T lymphoblastic leukemia cell line & RAW 264.7 murine macrophage cell lysate respectively. Cell growth inhibition was observed by using MTT assay. DMBE significantly decreased cell count and caused apoptosis, as confirmed by light, fluorescence, confocal microscopy and by flow-cytometric analysis using Annexin-V FITC and propidium iodide (PI). MOLT-3 cells showed DMBE induced cell cycle arrest, and apoptosis was mediated through mitochondrial intrinsic pathway involving the release of cytochrome c into the cytosol and activation of caspase-9 and caspase-3. DMBE induced changes in cellular ROS production upon stimulation with DCFDA.

Keywords: *Diospyros melanoxylon* Bark, Leukemia, Apoptosis, Caspase, Antioxidant.

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INTRODUCTION

One of the major health problems to have emerged in the recent times is haematological malignancy. Leukemia is a malignant leukocyte cancer characterised by the overproduction of large number of immature blood cells that enter the peripheral blood^{1,2}. The mainstays of leukemia treatment for adults have been chemotherapy, radiation therapy, and stem cell transplantation. New therapeutic approaches are important to improving outcomes. Medicinal plants have a long history of being used to cure numerous illnesses and different forms of cancer³. Bioactive compounds derived from plants are considered as renewable sources of anti-leukemic agents due to their diversity and availability. Findings on the anti-proliferative effects of pure compounds and crude extracts from plants against leukemia have questioned their ability to replace established drug treatments for leukemia^{4,5}.

Both, unripe, as well as ripe fruits have been used in folk-medicine by tribal communities. The leaf, bark and fruit of *D. melanoxylon* have also been used for traditional medicines^{6,7}. Leaves are used as styptic, in the treatment of scabies and old wounds, and as laxative and carminative medicine⁷. *Kendu* fruit also helps in stomach disorders. The dried fruit powder is used as carminative and astringent agent and is useful in treating urinary, skin and blood

diseases. This may be attributed to high tannin content (15-23 %) of the fruit⁸. *Kendu* bark also contains various steroids, alkaloids, glycosides, proteins, phenolic compounds and tannins^{4,5}. The seeds can be intoxicating; they have been prescribed in India as a cure for mental disorders, nervous breakdowns and palpitations of the heart. The fruits have a cooling and an astringent effect. The dried flowers are reportedly useful in urinary, skin and blood diseases. The bark is serves as astringent and its decoction is used to treat diarrhoea⁹.

MATERIALS AND METHODS

Chemicals and Reagents

RPMI 1640 (Gibco), fetal bovine serum (FBS), trypsin (Gibco, USA), penicillin-streptomycin (Biowest, Germany), gentamycin (Nicholas, India), HEPES, L-glutamine, MTT, acridine orange, ethidium bromide, agarose (Puregene), proteinase k (SRL), Annexin-V FITC (apoptosis kit), RNase, propidium iodide, Caspase-3 and 9 (Sigma), DMSO (dimethylsulphoxide). Phenol, Chloroform (Qualigens Fine Chemicals), Isoamyl alcohol, Agarose, (Sisco R.L. Pvt Ltd), Sodium bi-carbonate, DCFDA & Ethanol (Merck) All other chemicals were of analytical grade were procured locally.

Cell culture

MOLT-3 and RAW264.7 cells (Macrophage cells) was obtained from the National Facility for Animal Tissue and Cell Culture, Pune, India. The cells should be handled under laboratory containment level 2 conditions. The MOLT-3 cells were cultured and routinely maintained in RPMI-1640 medium. The medium was supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100µg/ml). Cell lines were maintained in a humidified incubator with 5% CO₂ and 95% air at 37°C. It was sub-cultured weekly at an initial density



of 1×10^5 cells/ml and maintained. RAW 264.7 cells are adherent in nature. During sub culturing of the cells this adherent property can be diminished by adding 1x Trypsin solution in the cell.

Extraction and Preparation of Test Sample

The bark of *Diospyros melanoxylo*n Roxb. was collected from the tribal jungles of Ranchi. The plant was identified by Indian Botanical Garden, Howrah, India, where a voucher specimen was kept. One kg of *Diospyros melanoxylo*n bark was sun-dried, ground and soaked in 3 l of 50% aqueous methanol for one week at room temperature (28-34°C) with occasional shaking. The supernatant was filtered and to the residue 1 l of 50% aqueous methanol was added and kept for another one week. The methanol portion in the whole supernatant was evaporated using a rotary evaporator and then it was lyophilized to remove the water. The solid dark brown residue (35 g) thus obtained was designated as 'Diospyros melanoxylon bark extract (DMBE) and was kept at 4°C. From 1 mg/ml stock solution of DMBE in phosphate buffered saline (PBS), different suitable concentrations like 25, 50, 100 and 200 µg/ml were used for different in vitro experiments.

Cytotoxicity study

Cell cytotoxicity was assessed by the MTT assay. 1×10^5 cells (MOLT-3 and RAW 264.7) were added to each well of a 96-well microtiter plate separately and grown in RPMI 1640 and supplemented with 10% foetal calf serum. Cancer cells were cultured in presence and absence of DMBE in a concentration of 25, 50, 100 and 200 µg/ml. Plates were incubated at 37 °C in a humidified 5% CO₂ incubator for 24hr. 20µl MTT (5 mg/ml) was then added in each well and allowed to incubate at 37 °C in 5% CO₂ incubator for 3 hrs. 100 µl DMSO was added to each well to dissolve the formazan crystal formed. The OD was recorded at 492 nm with microplate reader (Merck-MIOS Mini, Model No. 309). Growth inhibitory rate (%) was expressed as $\frac{1}{4} [1 - (\text{OD of treated} / \text{OD of control})] \times 100$ ¹⁰.

Toxicity study on RAW 264.7 cell line by MTT assay

RAW264.7 cells (1×10^5) were seeded in 96-well plates and incubated inside a CO₂ incubator for 24 hrs before treatment. Cells were treated with DMBE in doses of 25µg, 50µg, 100µg, 200µg for a period of 24hr at 37°C in a humidified atmosphere containing 5% CO₂ in air, untreated cells served as control. Cytotoxicity study was performed by MTT assay with slight modifications. 100 µl of the supernatant was removed, 20 µl of MTT (5mg/ml) was added to each well and 100 µl fresh media was then added to each well because of the adherent capacity of RAW 264.7 cell line. The plate was allowed to incubate for 3-4 hours at 37°C in 5% CO₂ incubator. 100 µl of DMSO was added to each well to dissolve the formazan crystal formed and the O.D values were recorded at 492 nm¹¹.

Determination of IC₅₀ value

The 50% of inhibition concentration (IC₅₀) value of DMBE in MOLT-3 cells was determined using different concentrations (25, 50, 100, 200 µg/ml) of the extract at 24 hr. The respective concentration of extract was converted to their respective log dose. The percentage of cell death was converted to Probit value in respective dose concerned. The graph was plotted with the different log dose of the extract against the respective Probit value. The IC₅₀ value was noted. The results are expressed as IC₅₀ (mean values ± SD, standard deviation) of at least two independent experiments, in six repetitions.

Light & Fluorescence microscopy

MOLT-3 cells (1×10^6) were treated with DMBE in a sterile cover slip and incubated for 24 hrs at the conditions mentioned above. The cells were then washed twice with PBS and observed under a light microscope at a magnification of 40X. MOLT-3 cells were treated with DMBE (IC₅₀ dose) in a sterile cover slip and incubated for 24 h at the specific conditions mentioned above. After washing with cold PBS, the cells were treated with 10 µl of staining solution containing acridine orange and ethidium bromide in 1:1 ratio and immediately observed under a fluorescence microscope (Leica Fluorescence Microscope) at a magnification of 60X, excitation range–blue filter BP450-490, dichromatic mirror-510 and suppression filter-LP515¹⁰

Confocal microscopy

MOLT-3 cells (1×10^6) were treated with IC₅₀ dose of DMBE for 24 hr. The untreated control cells and DMBE 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 BESC treated leukemic cells under confocal laser scanning microscope (Leica TCS-SP2 system, Leica Microsystem, Heidelberg, Germany) installed with an inverted microscope (LeicaDM-7RB). 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-3 cells were treated with IC₅₀ dose of DMBE for 24 hrs then cells were resuspended in 500µl of lysis buffer (50 mM Tris- 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 1hr 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 transilluminator.



Annexin-V binding study

MOLT-3 (1×10^6) cells/ml were cultured in presence and absence of DMBE (IC_{50} dose) for 24 hr. The cells were then washed with phosphate buffer saline and centrifuged at 1500 rpm at 4 °C. The cell pellet was washed twice with 1X Annexin–Hepes buffer and resuspended in 100 ml of this buffer. Annexin-V antibody (final concentration of 0.2 mg/ml) was added to this cell suspension and incubated for 30 min in ice in the dark room. Then it was washed again with 1X Annexin–Hepes buffer and fixed with 1% paraformaldehyde at 4 °C. The cells were analyzed within 3–4 h by flow cytometry (Becton Dickinson, FACS Calibur) with Cell Quest software. Flow cytometer was set for collecting data of 10,000 cells in each group¹².

Cell cycle analysis

For the analysis of cell cycle phase distribution of DNA, respective control, standard and DMBE-treated cancer cells (1×10^6 each) were washed with PBS after 24 h of DMBE treatment (IC_{50} dose) and fixed in 70% ethanol and stained with propidium iodide (100 mg/ml in 0.1% sodium citrate containing 0.1% Triton X-100) after RNAase (10 mg/ml) treatment. The cell cycle distribution was determined by flow cytometry (Becton Dickinson, BD LSR TM three-laser Flow Cytometer) using Cell Quest software. Fluorescence detector equipped with 488 nm Argon laser light source and 623nm band pass filter. Total of 10,000 cells were acquired for analysis¹².

Caspase-9 activity

The assay was performed using a Caspase-9, Apoptosis Detection, Colorimetric BioAssay Kit (US Biological) according to the manufacturer's protocol. MOLT-3 cells (1×10^6) were treated with the IC_{50} dose of DMBE 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 one min, 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 10 mM DTT) was added to each sample. 5 μ l of LEHD-pNA substrate (4 mM) (200 mM 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 done¹⁰.

Caspase-3 activity

The assay was performed using a Caspase-3 Assay kit, Colorimetric (Sigma) according to the manufacturer's protocol. MOLT-3 cells (1×10^6) were treated with DMBE (IC_{50} dose) for 24 h. The untreated control and the treated cells were pelleted down by centrifugation at 600 x g for 5 min at 4 °C. Supernatants were removed and the cell pellets were washed with 1 ml of PBS. The cells were again centrifuged and the supernatants were removed completely. The cell pellets were suspended in 100 μ l of 1X lysis buffer (50 mM HEPES, pH 7.4, 5 mM CHAPS, 5 mM DTT) and incubated on ice for 20 min. The lysed cells were

centrifuged at 20,000 x g for 15 min at 4 °C and the supernatants (cell lysates) were analysed for the caspases-3 activity according to the manufacturer's protocol. Cell lysates were incubated with 2 mM Caspase-3 substrate (Ac-DEVDpNA) in 1X assay buffer (20 mM HEPES, pH 7.4, 2 mM EDTA, 0.1% CHAPS, 5 mM DTT) for 90 min at 37 °C. The absorbance was read at 405 nm and the results were calculated using a p-nitroaniline calibration curve¹⁰.

Anti-oxidant Study

During apoptosis there is an increased ROS generation in the mitochondria that leads to oxidative stress and cellular damage. To examine the effect of DMBE (IC_{50} dose) on generation of ROS in MOLT-3 cells, CMH2DCFDA – lipid soluble, membrane permeable non-fluorescent reduced derivative of 2,7-dichlorofluorescein was used based on the evidence that the acetate groups of CMH2DCFDA are removed by esterase cleavage intracellularly to produce hydrophilic, non-fluorescent dye Dichlorodihydro fluorescein (DCFH2) which is subsequently oxidized by ROS to form a highly fluorescent product Dichlorofluorescein (DCF). Thus, the fluorescence generated is directly proportional to the quantum of ROS generated. The effect of IC_{50} concentration of DMBE on ROS generation was measured in the MOLT-3 leukemic cell. Post treatment with DMBE, the cells were washed with PBS, resuspended in PBS and incubated with CMH2DCFDA (5 mM in PBS) for 30 min at 37 °C. Subsequently, cells were washed and again resuspended in PBS. DCF fluorescence was determined by flow cytometry at an excitation wavelength of 488 nm and an emission wavelength of 530 nm. Mean fluorescence intensities (MFI) were obtained using the FACS Diva software^{13,14}.

Statistical analysis

Statistical Analysis All data are represented as arithmetic mean \pm S.E.M. Statistical analysis was done by Student's t-test. A probability value of less than 0.05 was chosen as the criterion of statistical significance.

RESULTS

Cytotoxicity study

DMBE at concentrations of 25, 50, 100 & 200 μ g/ml significantly inhibited the growth of MOLT-3 cells compared to that of the control cells at 24 hrs in a concentration dependent manner (Fig.1). There was a significant concentration – dependent reduction in the O.D values, along-with corresponding percentage inhibition in the DMBE treated cells. These findings provide proof for the cytotoxic nature of DMBE. However, DMBE exhibited insignificant cytotoxicity on RAW264.7 murine macrophage cells, rather in consecutive doses up to 50 μ g there was an increase in RAW 264.7 O.D. at 492 nm as observed by MTT assay suggesting DMBE selectively acted upon the cancer cells. The IC_{50} value of DMBE was found to be 52.8 μ g/ml. All the further experiments were done with the IC_{50} dose.



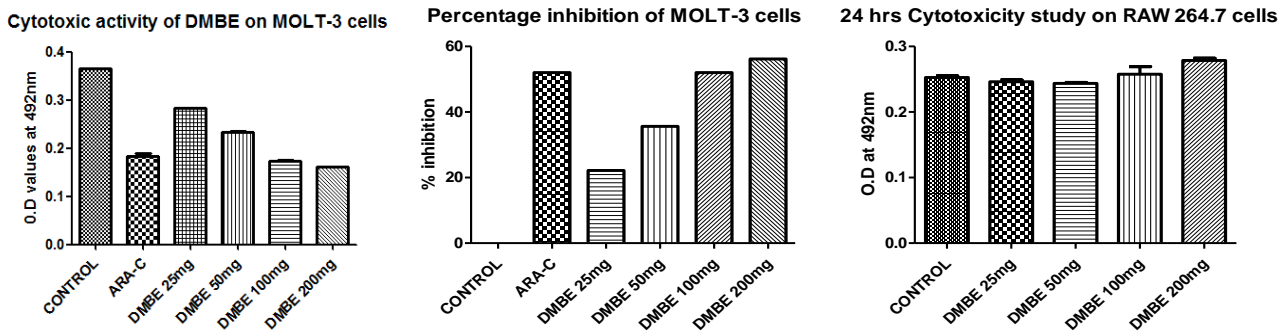


Figure 1: The histogram showed significant reduction in MOLT-3 cells post- treatment with DMBE compared to the untreated control cells in a concentration dependent manner. Ara- C was the standard drug used. Data are mean \pm SEM of triplicate determination. *, ** denote a significant decrease in O. D. at 492 nm from control values $P < 0.05$, $P < 0.01$ respectively. (B) Histogram shows the insignificant cytotoxic effect of DMBE on RAW264.7 murine macrophage cells.

Light & Fluorescence Microscopy Study

Light microscopic images of MOLT-3 cells treated with DMBE (IC_{50} dose) revealed the presence of fragmented nuclei as compared to the untreated control cells with intact nuclei. MOLT-3 cells treated with DMBE and stained with both acridine orange and ethidium bromide confirmed the presence of early and late apoptotic cells 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 apoptosis.

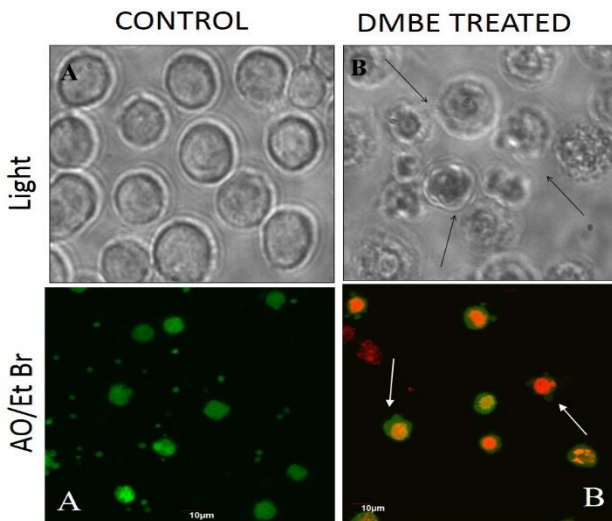


Figure 2: Light & Fluorescence microscopic images showed untreated control (A) cells were with intact nuclei and gave bright green fluorescence whereas DMBE treated (B) MOLT-3 cells were intense orange-red fluorescence showing signs of apoptosis.

Confocal Microscopic Studies

DMBE induced apoptotic changes in the MOLT-3 leukemic cells after 24hr of treatment showing chromatin disintegration and formation of apoptotic bodies whereas the untreated control cells were with intact nuclei (Fig. 3).

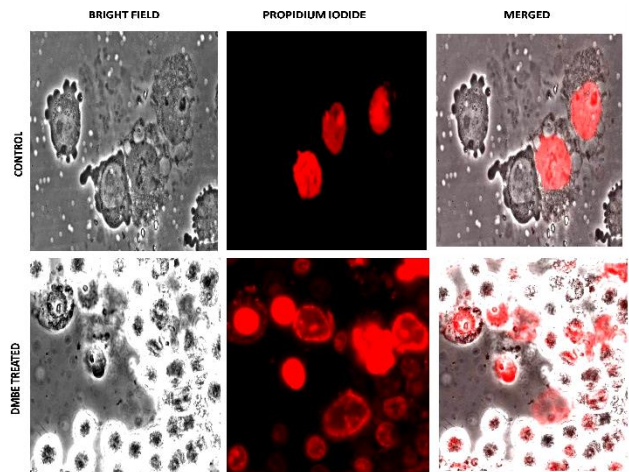


Figure 3: Confocal microscopic images of MOLT-3 cells showed apoptotic changes like nuclear disintegration and formation of apoptotic bodies for DMBE treated cells stained with propidium iodide whereas untreated control cells showed intact nuclei.

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-3 cells. Whereas treated cells showed fragmented DNA of MOLT-3 cells on agarose gel indicating the apoptotic activity of DMBE.



Figure 4: Lane 1 represents MOLT-3 control cells, lane 2 Ara- C treated cells and lane 3. DMBE treated cells.

Detection of apoptosis by flow cytometry

In late stages of apoptosis, cells split to form apoptotic bodies. Each apoptotic body contains only part of the original cell's DNA content. When stained with PI, this population is known as the sub-G1 population and is characterized by having a DNA content of less than 2n chromosomes. In addition, apoptotic cells demonstrate specific morphological changes such as chromatin condensation and plasma membrane blebbing. These changes cause the cell to be more granular and larger in size when analysed by FACS. 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. Flow cytometric data analysis for quantification of apoptosis after 18 h of treatment with desired dose of DMBE revealed a 2.5-fold increase in the cell population (15.9 %) in upper right quadrant as compared to 6.1% which thereby implies early apoptosis in the MOLT-3 cells.

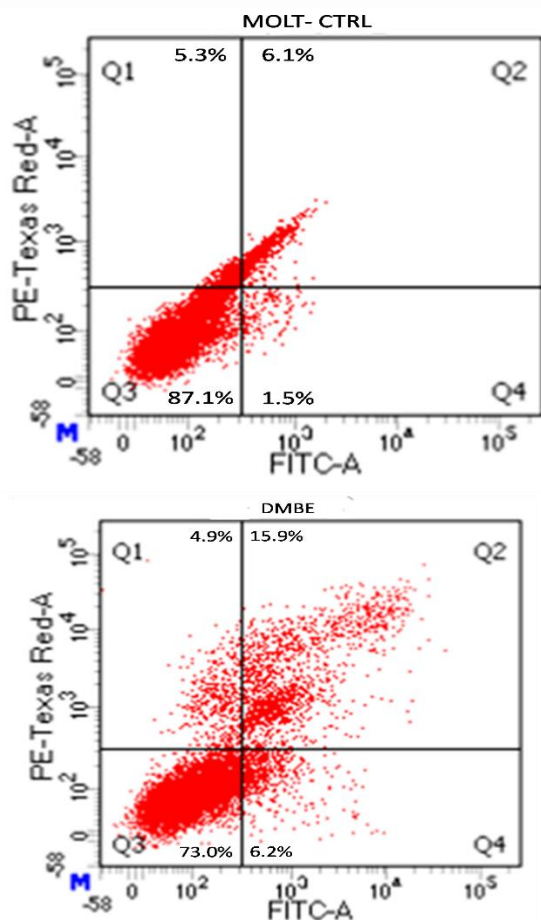


Figure 5: Detection of apoptosis by flow cytometric analysis of MOLT-3 cells in both control and DMBE treated cells respectively, after 18 hr. staining was done with Annexin V FITC and propidium iodide. Dual parameter dot plot of FITC-fluorescence (x-axis) vs. PI-fluorescence (y-axis) showed logarithmic intensity.

Detection of Cell Cycle Arrest by Flow Cytometric Analysis

Flow cytometric analysis after 18 hrs treatment of MOLT-3 lines with DMBE at IC50 dose, depicted changes in the G0/G1 phase and G2/M phase DNA content increased by 1 fold in treated cells than that of control (32.5% against 31.5%) in G0/G1 phase, as well as in G2/M phase (15.3% against 10.6%). All data are represented as arithmetic mean \pm S.E.M.

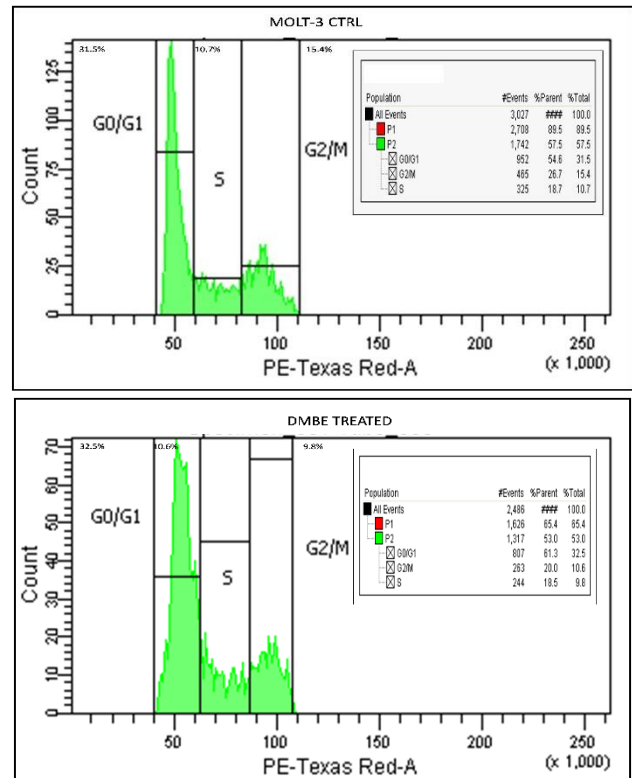


Figure 6: Flow cytometric analysis of cell cycle phase distribution on MOLT-3 cells in both control and DMBE treated cells, respectively after 24 h treatment. Histograms represented various contents of DNA with the actual number of cells (x-axis denotes the fluorescence intensity of PE-Texas red and y-axis denotes count).

Caspase-9 and 3 activity

Caspase -9 is one of the initiator caspases linked to the mitochondrial death pathway. To unravel the pathway through which cell death occurred, Caspase-9 assay was performed in the MOLT-3 cells upon treatment with IC50 dose of DMBE. There was a significant 4-fold increase observed in the DMBE treated cells as compared to the control (Fig. 7) supporting the fact that apoptosis induced by DMBE might be mediated by the intrinsic pathway. Intracellular caspase 3 activation is a key stage in the apoptotic pathway. Hence, we tested the effect of treatment with our plant extracts on intracellular caspase 3 enzymatic activity. In these experiments, total caspase 3 activity from the entire cell population was measured. Since caspase 3 activity rises while cells die and cell numbers drop, it was essential to normalize caspase 3 activity to the number of cells, to obtain more accurate results. Therefore, identical samples were analysed simultaneously for cell

viability (Fig.7). The results here are expressed as the 5-fold increase in caspase 3 activity in treated cells compared to cells added with DMSO/PBS (at the same final concentration as in the plant extracts; control cells).

Antioxidant Study

Induction of oxidative stress by DMBE treated MOLT-3 cells was evaluated by determination of the reactive oxygen species. Treatment with IC₅₀ dose of DMBE induced intracellular ROS generation as detected by DCFDA as shown in Fig. 8. DCFDA is cell permeant fluorogenic dye that undergoes deacetylation by cellular esterases. It generates fluorescent compound in presence of reactive oxygen species (ROS). The number of free radicals increases with generation of more oxidative stress which on the other hand generates more fluorescence as evident from Fig.9 where DMBE treated cells produced more fluorescence as compared to control.

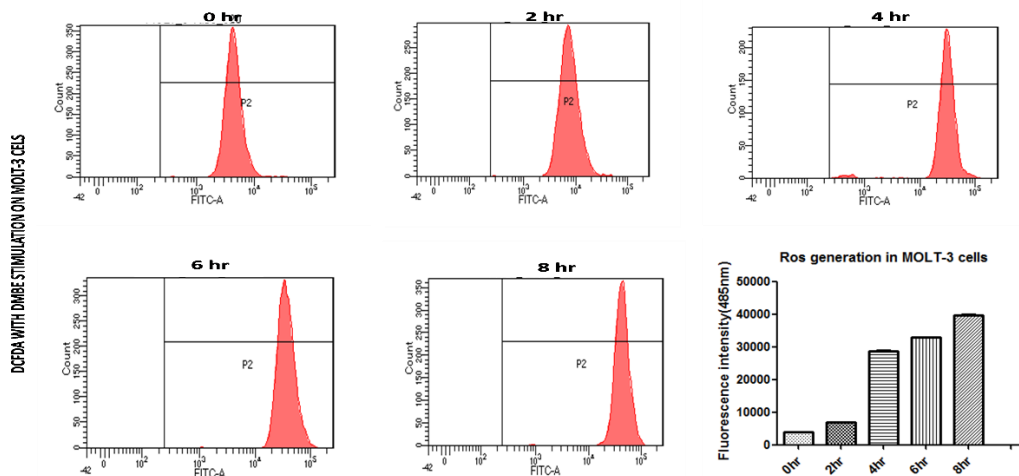


Figure 8: Flow cytometric analysis of ROS generation by DCFDA in MOLT-3 cells after treatment with IC₅₀ dose of DMBE. Histogram display of DNA content (x- axis, PI- fluorescence) vs. count (y-axis).

DISCUSSION

With the growing number of newly diagnosed cases of all forms of cancer globally, and in particular, haematological malignancies, there is still a strong need for novel agents that may treat or eliminate cancer. For many reasons, medicinal plants have been used to cure diverse illnesses for centuries, due to the enormous chemical diversity and biological selectivity of the bioactive compounds. In this study we demonstrated that *D. melanoxylon* bark extract has anticancer activity against MOLT-3 cell line causing these cancerous cells to die via apoptosis. DMBE also exerted positive effect on the RAW 264.7 murine macrophage cell line with no significant toxicity, suggesting that DMBE preferentially acts on MOLT-3 leukemic cells. Cytotoxic activities of DMBE was supported by the observations in cell morphology. Light and fluorescence microscopic images clearly showed nuclear disintegration of DMBE treated leukemic cells compared with that of the untreated control cells when stained with acridine orange and ethidium bromide. Untreated control cells showed bright green fluorescence as live cells with

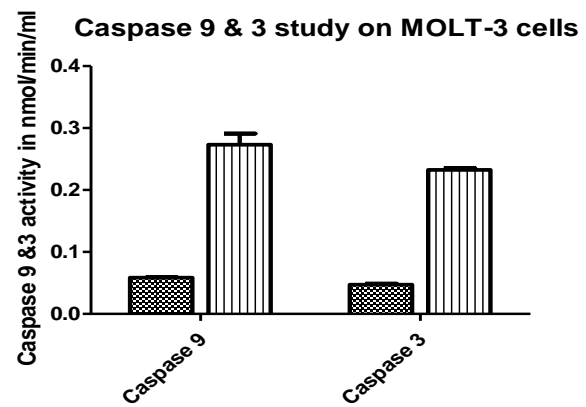


Figure 7: Caspase-9 and Caspase-3 activity in MOLT-3 cells upon treatment with DMBE. The p value was < 0.001 in bars with no SE.

intact membrane excluded ethidium bromide and allowed only acridine orange to permeate through. On the contrary, DMBE treated cells showed more intense orange-red fluorescence and reduced green fluorescence since apoptotic and necrotic cells could not exclude the dyes and gave a combination of orange-red and green fluorescence. Apoptotic activity of DMBE was further evidenced from the confocal microscopic images of the treated leukemic cells when compared with the untreated control cells. Post treatment with DMBE, MOLT-3 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. Dual staining with annexin V FITC and propidium iodide in dot plot assay made it possible to identify live, early apoptotic and late apoptotic cells^{16,17}. Experiments showed increased number of cells in the early and late apoptotic stage after treatment with DMBE. Cell cycle analysis revealed that treatment with DMBE arrested the MOLT-3 cell population in the G₀/G₁ and G₂-M phase and phase of cell cycle. Mitochondria-mediated apoptosis occurs in response to a

wide range of death stimuli, including activation of tumor suppressor proteins (such as p53) and oncogenes. The intrinsic pathway is governed by the translocation of cytochrome c into the cytosol. Cytochrome c triggers caspase-9 activation initiating a downstream caspase cascade through the complex formation with Apaf-1, dATP, and pro-caspase-9 in the cytosol, which ultimately lead to the activation of the executioner caspase-3 and finally cell death^{15,18}. Caspase-9 and caspase-3 assays showed increase in the activities of caspase-9 and caspase-3 respectively post-treatment with DMBE in MOLT-3 cells indicating that the cytochrome c release was a preceding event for the activation of the mitochondria mediated caspase cascades. Based on these findings, it can be suggested that intracellular signaling mediated the cytotoxic effect of DMBE, which may be based on a mechanism involving mitochondrial ROS upregulation.

CONCLUSION

We successfully evaluated the cytotoxic and anti-oxidant activity of *Diospyros melanoxylon* bark extract on the MOLT-3 and RAW 264.7 cells. The present investigations confirmed DMBE did not exhibit any cytotoxicity on RAW 264.7 macrophage cell lines, indicating selective cytotoxicity only upon the cancer cells. It is well reported that plant extracts and their bioactive compounds effectively kill leukemia cells and have produced similar effects in animal studies too. Phytomedicines exert different mechanisms of action including suppressing proliferation; causing cell cycle arrest; apoptosis; along-with dose and time-dependent damage to DNA. DMBE caused cell death via the activation of the mitochondrial membrane caspase cascade pathway and cell cycle arrest. Also ROS by the MOLT-3 cells upon treatment with DMBE triggered oxidative stress-induced cancer cell death. The minor fruit tree being rich in nutritional, medicinal and processing qualities can play a very significant role in the livelihood security of the rural communities through enhanced household income, employment generation and environmental medicinal.

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