Summary and Conclusion
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Reactive oxygen metabolites are generated either by the transfer of radiant energy to the oxygen molecule or by its stepwise reduction. Usually, free radicals are generated by exposure of tissue to ionizing radiation and ultraviolet light. Cancer cells can generate large amounts of hydrogen peroxide and this, if it occurs in vivo, may contribute to their ability to mutate and damage normal tissues, and moreover, facilitate tumour growth and invasion. Semecarpus anacardium Linn. nut milk extract (SA), a Siddha medicine was evaluated for its potential anti-mutagenic and anti-leukemic effects.

The leukemic cells possess a number of properties, which are biologically different from most of the host cells. Numerous biochemical and molecular alterations occur in the leukemic conditions leading to the derangement of cellular components and functions in the body. Mitochondria are involved either directly or indirectly in many aspects of altered metabolism in leukemic cells and several notable difference between the mitochondria of normal versus transformed cells have been discovered. Several biochemical and pathological derangements were observed in the spleen and liver of the leukemic mice.

The balance between proliferation and apoptosis is crucial in determining the overall growth or regression of the tumour. Apoptosis positively modulates the overall growth or regression of the tumour. Apoptosis was positively modulate correlated with duration of the treatment
and the inhibition of leukemic cells, but inversely with bcl-2 expression during treatment. Thus, it is possible to delineate the biology of leukemia at the molecular and biochemical levels by examining apoptosis and its control and regulation.

The chemopreventive/therapeutic efficacy of SA was observed in both in vivo experimental Bcr-Abl+ leukemia induced BALB/c mice and in vitro 12B1 murine leukemic (Bcr-Abl b3a2 fusion gene) cell line. The findings of the study have been summarized below.

- SA is known for its pharmacological activity and it has been shown that the extract can be used as an effective and safe antimutagenic agent. It possesses antioxidant property and can prevent the strand break formation in supercoiled DNA. It is an excellent free radical scavenger which protects cells from UV radiation induced DNA damage.

- Histopathological examination showed the infiltration and morphological changes in bone marrow, blood, spleen and liver indicative of induction of acute leukemia analogous to CML in blast crisis. Administration of SA, revealed normal morphology of bonemarrow, blood, spleen and liver which confirmed the anti-carcinogenic potential of the drug.

- Leukemic mice showed a significant decrease in the body weight and internal organ weight changes were observed in the leukemic condition. Considerable tumour progression and significant body
weight loss were observed in untreated mice. Administration of SA showed a significant regression of tumour and significant increase in the body weight.

The levels of hematological parameters such as RBC and Hb were decreased and WBC were elevated in leukemia induced animals, whereas the deranged hematological indices were reverted back to near normal states on treatment with the drug (SA).

DNA damage is sensitive marker in the carcinogenesis. The levels of nucleic acid have been estimated. In untreated animals, the levels were found to be increased while treatment with SA reversed the nucleic acid content to near normal levels.

Glycoprotein serve as reliable classical marker and an indicator in the progression of tumour in the malignant condition. The levels of glycoprotein components were elevated in the diseased conditions and brought back to near normal levels on drug (SA) administration indicating its positive protective effect on the membranes and bringing back the membrane integrity. This shows that the drug has the capacity for rejuvenating and bringing back the original structure and function of the damaged cell membrane and the membrane of other subcellular organelles like mitochondria too.

Lysosomal enzymes play a significant role in the breakdown of cells and intracellular substances thereby enhancing tumour invasion, the activities of lysosomal enzymes were significantly increased in
diseased animals. The administration of SA significantly decreased the enzyme activities. This could be due to the stabilizing property of the drug on lysosomal membrane.

The damages inflicted by free radicals to cellular macromolecules like lipids and proteins play a crucial role in malignant conditions. The levels of lipid peroxides were significantly increased in diseased condition. Administration of SA brought the levels of lipid peroxides to near normal level suggesting that SA protects by controlling the free radical production.

The damages inflicted by free radicals to cellular macromolecules like lipid, proteins and nucleic acids play a crucial role in the pathogenesis of cancer. The levels of DNA-strand breaks and DNA-protein cross links were found to be elevated in leukemic mice. Treatment with SA brought the levels of these macromolecular damages to a close limit to normal conditions suggesting that the drug protects by inhibiting free radical production on account which the macromolecular damage is also reduced sizably.

Membrane damage is a basic feature of the malignant cells. The membrane bound ATPase activites namely, Na\(^{2+}/K^+\)-ATPase; Mg\(^{2+}\)-ATPase and Ca\(^{2+}\)-ATPase were decreased indicating the severity of the disease. The activities were restored to near normal levels upon drug treatment, indicating its membrane stabilizing action.
Energy metabolism plays an essential role in leukemia. Elevated activities of glycolytic enzymes and decreased activities of gluconeogenic and Kreb’s cycle enzymes were observed in the spleen and liver mitochondria of the leukemia bearing mice. On administration of the drug (SA), the activities were reverted back to near normal levels, showing a positive trend on treatment with the drug i.e. leukemic condition driven back to normal condition. On SA treatment the activities of energy metabolism enzymes were significantly increased, thereby preventing cancer cachexia.

Decreases in the activities of ETC complexes were observed in both spleen and liver of leukemia bearing mice. The significant elevation in the activities of ETC complexes was observed in SA treated mice. This effect of the drug may be attributed to its effect on improving the electron transport and subsequent energy production.

Transmission electron microscopic observations of spleen of leukemia bearing mice revealed various derangements and modification of the cellular components. Imatinib mesylate and SA treated mice restored normal morphology of the spleen and increased the mitochondrial damage, ultimately leading to apoptosis.

Drug induced apoptosis was studied in vitro by using 12B1 murine leukemic cells at a dose of 60 μg and time interval of 24 h. Assesment of antiproliferative effect of the drug on $^3$[H] -
thymidine incorporation in leukemic cells showed marked inhibition of proliferation at a dose of 60 μg

Fluorescent microscopic pictures of drug treated leukemic cells showed nuclear fragmentation, typical findings of apoptotic cell death. The drug induces apoptosis by Bcl-2 suppression, Bax activation, cytochrome c release and caspases (caspase-9 and 3) activation

The drug SA also inhibits the expression of Bcr-Abl gene. *in vivo* as well as *in vitro*