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## A novel semi-synthetic analog of betulinic acid induced apoptotic cell death in HL-60 cells via mitochondrial disruption and perturbance in DNA repair mechanism

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In light of the scientific promise of chemoprevention, there is an overwhelming need to develop new chemopreventive agents that are both effective and safe. Natural compounds of diverse structures have been considered prototypes, leads or heads of series. Approximately half of the drugs currently used in the clinic are derived from natural products and in the case of cancer this proportion surpasses 60%. The modulation of signalling pathways that are deregulated in cancer offers a promising strategy for the discovery of novel anti-cancer therapeutics. In the present study, we examined the effect of a novel cyano derivative of betulinic acid (CBA), to induce apoptosis in a panel of human cancer cell lines representing diverse cancers. Using CBA we observed the appreciable IC50 values ranging from 1 μM to 7.1 μM in various cancer cells used in the present study. However, HL-60 cells representing leukemia was found to be most sensitive with $IC_{50}$ value of 1 μM 12 μM and 24 μM after 48 hr, 24 hr and 12 hr treatments respectively. The mechanism of cell death involved the increased sub-G0 DNA fraction, enhanced annexin V/FITC binding of the cells, DNA fragmentation, chromatin-condensation, formation of apoptotic bodies and morphological changes in the HL-60 cells. It was also observed that the CBA induced substantial reactive oxygen species (ROS) generation which also forms important aspect of chemotherapeutic efficiency in cancer. A persistent high level of ROS was associated with decline of the mitochondrial membrane potential with simultaneous activation of caspase 8 and 9. Western blot analysis showed the poly ADP-ribose polymerase (PARP) cleavage also occurs on HBA treatment which therefore suggests its involvement in the functioning of DNA repair machinery. Furthermore, CBA inhibited DNA binding of NF-kB and caused nuclear cleavage of p65/Rel which demonstrates that NF-kB mediated gene expression is down regulated under such conditions. These findings indicated that CBA can be potential candidate that may be found useful in the management of human leukemia.

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## Antibacterial photodynamic therapy on *Staphylococcus aureus* and *Pseudomonas aeruginosa in-vitro*

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**Background:** Photodynamic therapy (PDT) involves the use of drugs or dyes known as photosensitizers, and light source which induces cell death by the production of cytotoxic reactive oxygen species (ROS). This principle of cell death can be utilized to kill bacteria *in vitro*. We propose the use of blue light emitting diodes and riboflavin as the light source and photosensitizer for *in vitro* killing of *Staphylococcus aureus and Pseudomonas aeruginosa.*

**Methods:** Circularly arranged 65-blue LED array was designed as the light source to fit exactly over 7cm culture plate. Riboflavin having non-toxic properties and nucleic acid specificity was used as a photosensitizer. Clinical isolates of *Staphylococcus aureus and Pseudomonas aeruginosa* were used in our study. Effect of PDT on viability on these species of bacteria was compared with control samples that included: samples untreated, samples treated with light only and samples treated with riboflavin only.

**Results:** Statistical analysis was done using one-way ANOVA test. PDT against *Pseudomonas aeruginosa and Staphylococcus aureus* was significantly ($p<0.05$) effective compared to all control samples.

**Conclusions:** Combination of blue LEDs and Riboflavin in PDT against these bacterial species has been successfully demonstrated *in-vitro*. Therefore, PDT has promising applications in the process of treating superficial wound infections.