ANTICANCER ACTIVITY OF NEOSETOPHOMONE-B, A FUNGAL SECONDARY METABOLITE, AGAINST HEMATOLOGICAL MALIGNANCIES

GRADUATE STUDENTS

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Background

- Cancer, one of the worlds serious health problem and is one of the prime cause for millions of death, and the incidence rate is increasing day by day. Chemotherapy, radiation, surgery are some of the standard therapeutic strategies for curing cancer. But this has been hindered due to some drawbacks related to poor water solubility and bioavailability of drugs, leading to up regulation of multi drug resistance and adverse side effects, natural products are used as an alternative form for the treatment of cancer. Fungi represent a rich and important source of anticancer agents and the secondary metabolites produced by these fungi have been used in wide range of applications. In this study we would like to investigate whether fungal secondary metabolite Neosetophomone B (NSP-B) has ability to control the proliferation and induce apoptosis in leukemic cell lines,K562 and U937.

Study Objective

- To establish whether Neosetophomone B mediated inhibition in cell viability is due to cell cycle arrest/ apoptosis in leukemic cells.
- To investigate whether cell apoptosis is mediated via intrinsic or extrinsic pathway.
- To evaluate the effects of Neosetophomone-B on anti-apoptotic genes.

Materials and Methods

- Cell culture: K562 and U937 cell lines were cultured in RPMI 1640 medium supplemented with 10 % (v/v) fetal bovine serum (FBS), 100U/ml penicillin, 100U/ml streptomycin at 37˚ C in a humidified atmosphere containing 5 % CO_2.
- Cell Proliferation Assay: K562 and U937 cell lines were treated with increasing doses of Neosetophomone-B for 48 hours and analyzed using CCK-8 assay to identify dose-dependency of Neosetophomone-B and IC_{50}.
- Apoptosis: Fluorescent Annexin V and Dead assay apoptosis detection assay was performed via Muse flow cytometry.
- Mitopotential assay: $1x10^6$ cells were treated with different doses of Neosetophomone-B for 48 hours and mitopotential measured by Muse flow cytometry.
- DNA Laddering: DNA were isolated from the cells treated with different doses of Neosetophomone-B and run in agarose gel electrophorosis and view under chemiDOC imaging system.
- Western blot: K562 and U937 cells were treated with Neosetophomone-B for 48 hours, lysed and proteins were isolated. Equal amounts of protein were separated by SDS-PAGE, transferred to PVDF membranes and probed with specific antibodies.

Results

- Cell Proliferation Assay: NSP-B treatment on K562 and U937 cells cause a dose dependent Inhibition of cell viability was measured using Cell counting Kit-8. (Figure 1).
- Annexin V staining: NSP-B treatment induced apoptosis in leukemic cells and measured using Annexin V staining.(Figure 2).
- Immunoblotting Techniques: NSP -B induces the activation of both intrinsic and extrinsic apoptotic pathways. (Figure 3).
- DNA laddering Assay: NSP-B induces apoptosis in K562 and U937 cell lines by detecting the DNA fragments ,which further confirmed by probing with p-H2AX antibody, hallmark of DNA double strand break.(Figure 4).
- Measurement of Mitochondrial Membrane potential: NSP-B treatment on leukemic cells leads to loss of mitochondrial membrane potential.(Figure 5).
- Schematic representation of mode of action of NSP-B (Figure 6).

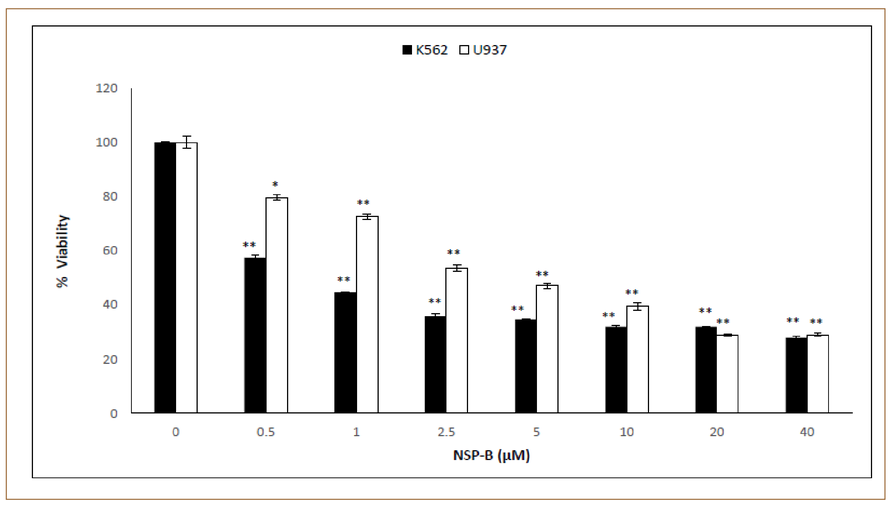

Figure 1:Treatment of NSP-B on leukemic cell inhibits the cell viability .

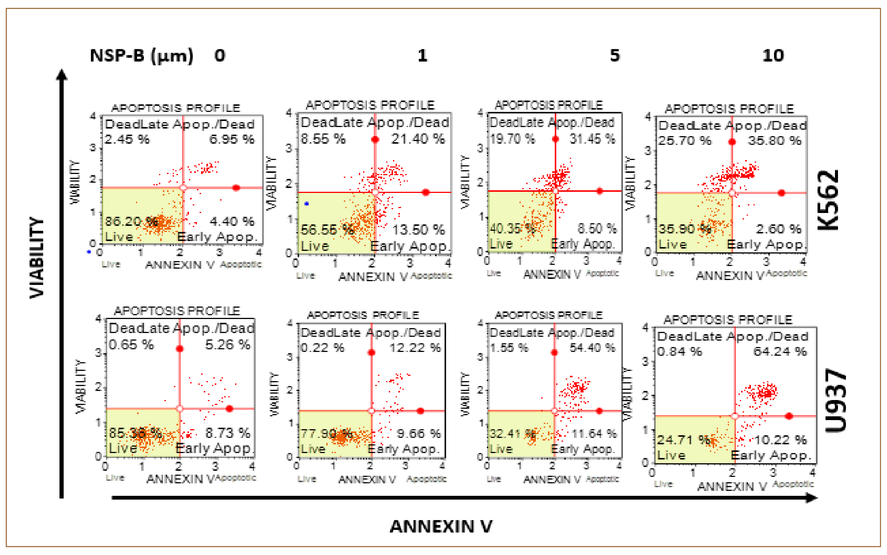

Figure 2:Neosetophomone-B induces apoptosis in K562 and U937 cell lines.

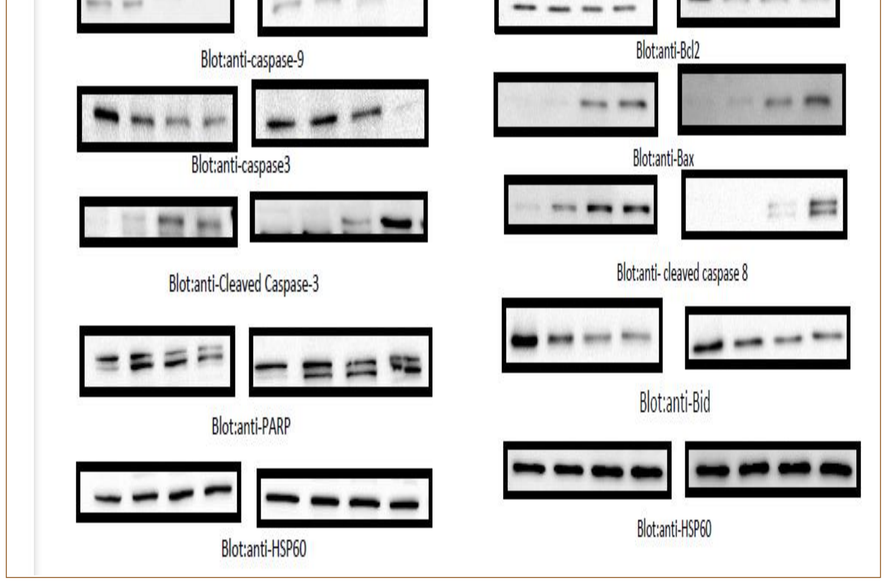

Figure 3:Neosetophomone-B induces both intrinsic and extrinsic apoptotic pathways in K562 and U937 cell lines

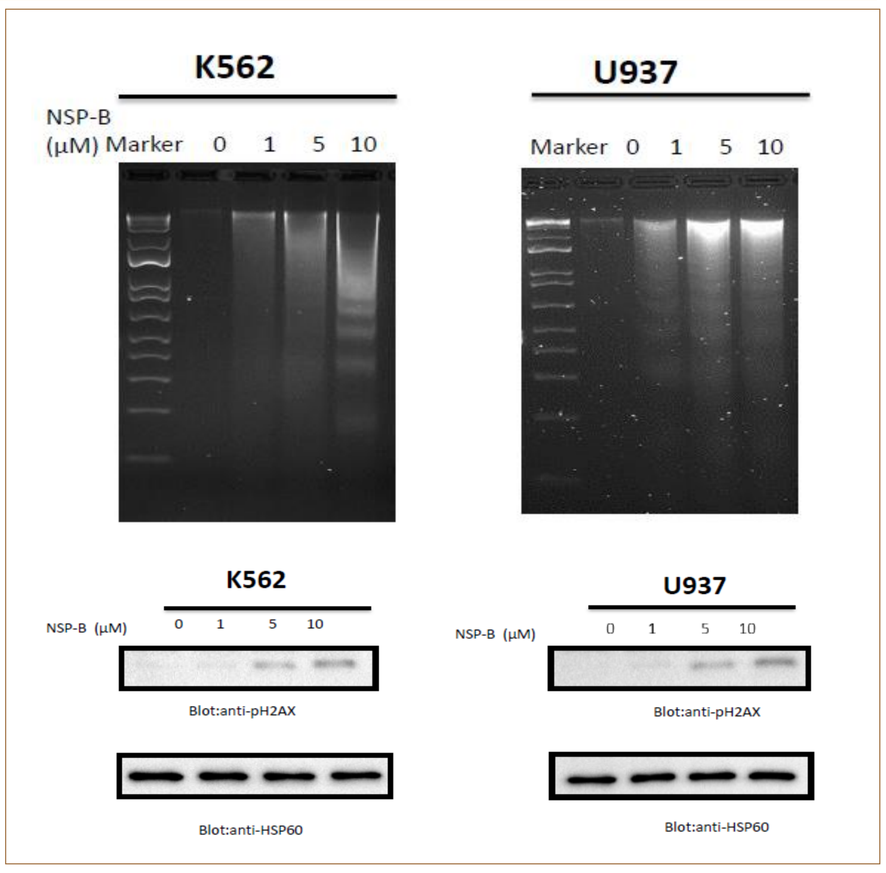

Figure 4:Neosetophomone-B induces apoptosis in K562 and U937 cell lines. Further confirmed by western blotting

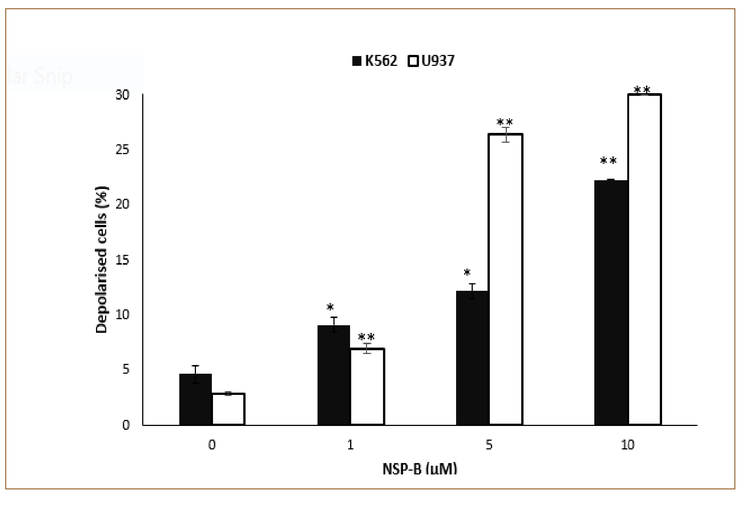

Figure 5:Treatment of NSP-B causes loss of mitochondrial membrane potential in leukemic cell lines

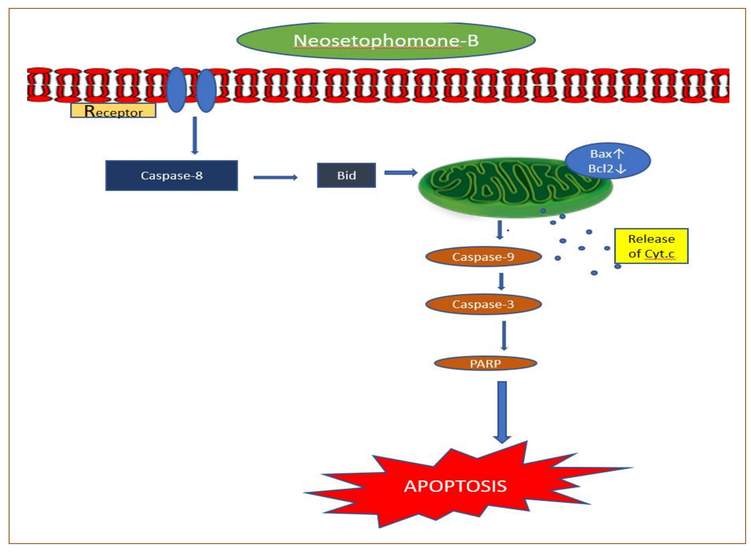

Figure 6:Schematic representation of mode of action of NSP-B.

Conclusions

- In this study, our results showed that NSP-B induced a dose-dependent cytotoxic effects in K562 and U937 cell lines .
- Our data showed an increased Annexin/PI staining supporting that NSP-B-mediated cytotoxic effects are due to induction of apoptosis.
- Western blotting confirmed that NSP-B can induced apoptosis via intrinsic and extrinsic pathway.
- DNA laddering results confirmed that NSP-B induced apoptosis by detecting the DNA fragments, which further confirmed by immunoblotted with P-H2AX, hallmark of DNA double strand break
- Loss of Mitochondrial Membrane potential confirmed that NSP-B induced apoptosis.
- Taken together, above results suggest that NSP-B may have a future therapeutic role in Leukemia and possibly other malignancies.

Limitations

- Ensuring enough supply of the compound as it is a pure natural compound with no other source and the isolation of the compound is a tedious process.
- Study was done only on the two leukemic cell lines, because of the time limit.
- Due to the current Covid-19 situation, some of the experiments are pending and kept for future studies.

Future Directions

- To investigate whether Neosetophomone-B can generate reactive oxygen species which subsequently triggers cell death.
- To investigate the genes or signature of genes involved in Neosetophomone B mediated anticancer effect by using Human Gene Chip assay and data analyzed by bioinformatics.
- To investigate whether co-treatment of Neosetophomone B with chemotherapeutic conventional drug will be able to cause apoptosis in a panel of cell lines.

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