An Investigation cytotoxic potential of silibinin mediated silver nanoparticles in HepG2 cells

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Abstract

Topic: An investigation of the cytotoxic potential of silibinin mediated silver nanoparticles in HepG2 Cells. **Aim:** To evaluate the anti-proliferative/ pro-apoptosis potentials of silibinin mediated silver nanoparticles in HepG2 cells. **Objective:** To investigate the cytotoxic potential of silibinin mediate silver nanoparticles HepG2. To investigate the pro-apoptotic potential of silibinin mediate silver nanoparticles in HepG2. **Conclusion:** SBN-SNPS- induces morphological changes related to apoptosis, cytochrome C dislocation to cytosol, and down regulates PCNA protein expression.

Keywords: HepG2 Cells, MTT assay, Silibinin

Introduction

Hepatocellular carcinoma (HCC) is the fifth common cancer in the world. It is a significant morbidity and mortality. Currently there are no promising drugs which are available for the treatment of HCC. Silibinin a Silybum marianum has antioxidant properties. Silibinin mediated silver nanoparticles are prepared and used against HepG2 cells. [2]

Silver nanoparticles are used in many commercial products in daily life. Exposure to nanosilver has hepatotoxic effects in animals. This study investigated the cytotoxicity associated with polyvinylpyrrolidone-coated nanosilver (23.44 ± 4.92 nm in diameter) exposure in the human hepatoma cell line (HepG2) and normal hepatic cell line (L02), and the molecular mechanisms induced by nanosilver in HepG2 cells.Nanosilver, in doses of 20–160 μg mL−1 for 24 and 48 h, reduced cell viability in a dose- and time-dependent manner and induced cell membrane leakage and mitochondria injury in both cell lines; these effects were more pronounced in HepG2 cells than in L02 cells.[2] Intracellular oxidative stress was documented by reactive oxygen species (ROS) being generated in HepG2 cells but not in L02 cells, an effect possibly due to differential uptake of nanosilver by cancer cells and normal cells. In HepG2 cells, apoptosis was documented by finding that ROS triggered a decrease in mitochondrial membrane potential, an increase in

cytochrome c release, activation of caspase 3 and caspase 9, and a decrease in the ratio of Bcl-2/Bax.[1] Furthermore, nanosilver activated the Fas death receptor pathway by down regulation of nuclear factor-κB and activation of caspase 8 and caspase.[2]

Materials, Method and Results

1. HepG2 saline will be produced from NCCS, Pune.
2. Cells will be supplemented with 10% FBS and 1% penicillin/ streptomycin and will be maintained at standard culture condition (37 degree celsius with 5% carbon dioxide).

Parameters:

1. Cell viability effect- MTT assay

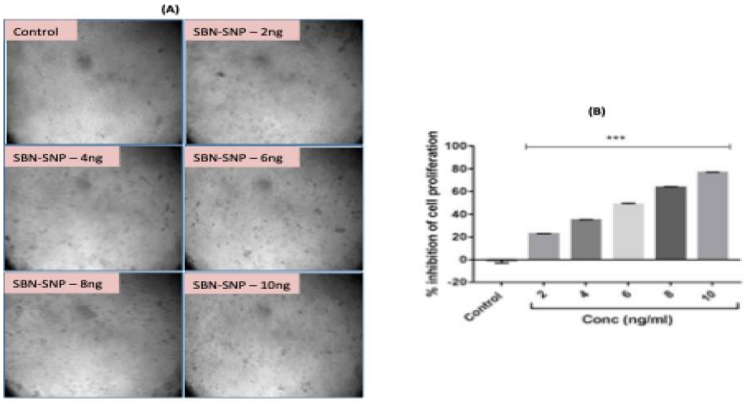

Figure 1. Silibinin mediated silver nanoparticles (SBN-SNPs) induced changes in the proliferation of HepG2 cells. A. Morphology of control and SBN-SNPs treated HepG2 cells. B. Cytotoxicity analysis by MTT assay. n=3, ***P < 0.001 vs control.

2. Morphological Changes- AO/ EO staining

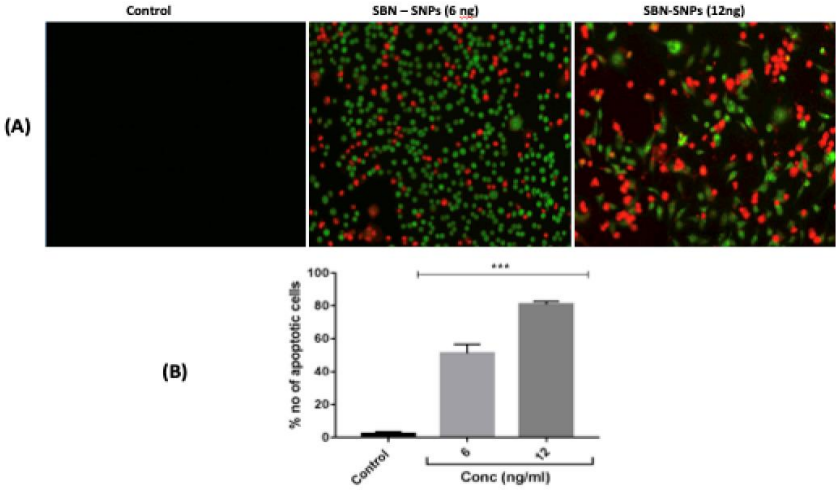

Figure 2. (A) Morphological analysis of apoptosis by acridine orange and ethidium bromide dual staining. (B). Quantification of apoptotic cells. n=3.***p < 0.001 vs control.

3. Investigation of NRF 1 expression – immunocytochemistry

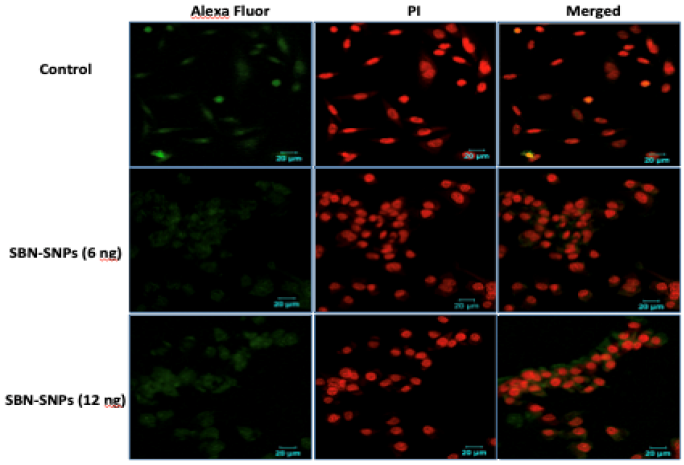

Figure 3. Immunofluorescence analysis of nuclear respiratory factor 1 (NRF-1) expression in control and SBN-SNPs in HepG2 cells. PI – Propidum iodide.

4. Investigation of cytochrome C expression- immunostaining

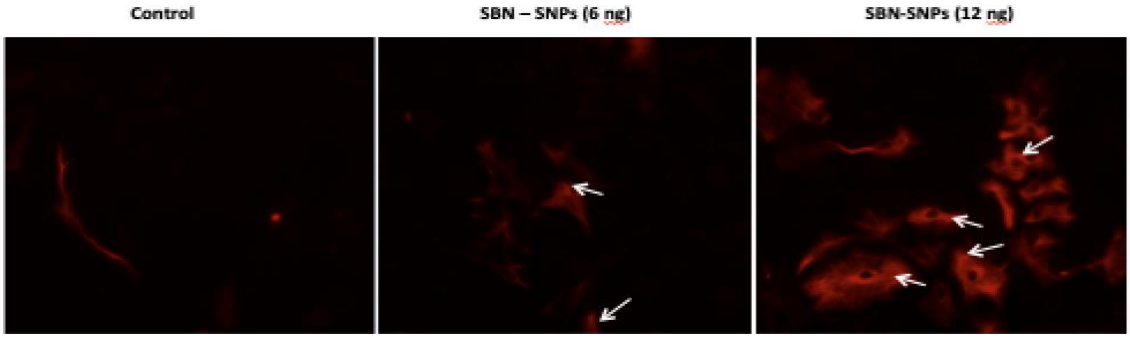

Figure 4. Cytochrome c dislocation analysis by immunoflourescance staining in control and Silibinin mediated silver nanoparticles (SBN-SNPs) treated HepG2 cells.

5. Analysis of PCNA protein expression- western blotting method

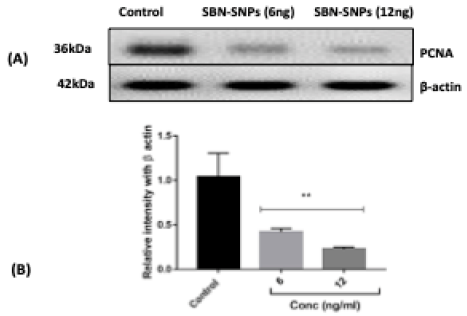

Figure 5. (A) Western blot expression of Proliferating cell nuclear antigen (PCNA). (B). Quantification of PCNA protein expression by densitometry analysis. n=3. ***p<0.01 vs control.

6. Statistical analysis- SPSS software (version 20.0)

Discussion

There is a sharp rise in the incidence of colon cancer due to absence of proper cure and the side effects related to the use of existing chemotherapeutics, has made it necessary to search for an effective therapeutic drug candidate. [1]Our results suggested that silibinin mediated silver nanoparticles could be cytotoxic to HepG2 cells. In a previous study, silibinin mediated silver nanoparticles caused a cytotoxic effect in SW480 colon cancer cell lines.[1] Further, studies have shown that anti-epileptic histone deacetylase inhibitors could induce cytotoxicity when treated with cancer cell lines (Abaza et al., 2014; Cornago et al., 2014; Tseng et al., 2017) and our current results are in consistent with the above investigations.[2]

Further, to find out the reason behind the cytotoxic potentials of silibinin mediated silver nanoparticles in HepG2 cells, we investigated the ROS expression because previous studies have shown that histone deacetylase inhibitors could induce cytotoxicity via ROS generation in a variety of cancer cells.For instance, sodium valproate induced ROS expression in glioblastoma cell line (Tseng et al., 2017) and even in colorectal cancer cell lines (Abaza et al., 2014). In light of the above investigations, it is to suggest that the cytotoxic potentials of silibinin mediated silver nanoparticles observed in this study could be attributed due to ROS accumulation in HepG2 cells. [1] When ROS levels are increased in cancer cells it induces oxidative stress which in turn damages the cell membrane and is consequently responsible for apoptosis (Ezhilarasan et al., 2019). Therefore, we analyzed apoptosis related morphological changes in HepG2 cells through dual staining.[2]

AO/EB fluorescent staining is usually performed to distinguish apoptosis-related morphological changes in cell membrane during the event of apoptosis(Liu et al., 2015).Apoptosis is characterized by distinct morphological changes that accounts for majority of cell death (Grilo and Mantalaris, 2019).Our results show early and late apoptotic cells upon silibinin mediated silver nanoparticles treatment. [1]silibinin mediated silver nanoparticles in low concentration induces late apoptosis as indicated by the presence of 7few red colored cells due to binding of EB with DNA in membrane damaged cells. However, higher concentration of silibinin mediated silver nanoparticles treatment led to the appearance of early (yellowish green) and late apoptotic cells (red) indicated the apoptotic inducing potentials.[2] These apoptosis-associated morphological changes could be attributed due to increased ROS expression in HepG2 cells upon silibinin mediated silver nanoparticles treatments.[1]

In order to ascertain whether ROS is responsible for the mitochondrial damage we analyzed the MMP. Studies have shown that enhanced ROS level could play central role in MMP loss(Zhang et al., 2015).[1]Previous evidences suggest that drugs incubated with cancer cells induce ROS with concomitant loss in MMP and apoptosis induction (Gillissen et al., 2017; Arumugam et al., 2016; Yan et al., 2015). In light of the above reports, it is to suggest that enhanced ROS expression in HepG2 cells upon silibinin mediated silver nanoparticles treatments could have responsible for the loss of MMP.[2]

Apoptosis is a programmed cell death and an important process to eliminate cells that are injured or damaged (Grilo and Mantalaris, 2019). Caspase 3 is a crucial mediator of apoptosis that initiates apoptotic chromatin condensation, formation of apoptotic bodies and DNA fragmentation(McIlwain et al., 2013).[2] Therefore, detection of caspase3 in cells is considered as an important method for apoptosis induced by a variety of apoptotic signals(Choudhary et al., 2015).Caspase 3 involves in intrinsic mitochondrial pathway of apoptosis. This pathway is activated by various factors including ROS that reduces MMP thereby responsible for the release of cytochrome c into cytosol.[1] In cytosol, cytochrome c activates the executioner caspase 3 in sequential manner to induce apoptosis (McIlwain et al., 2013; Gheena and Ezhilarasan, 2019).Our results also indicated that silibinin mediated silver nanoparticles treatments induced caspase3 expression in HepG2 cells and this could be the reason for the apoptosis inducing potentials of this drug.[2]

Conclusion

SBN-SNPS- induces morphological changes related to apoptosis, cytochrome C dislocation to cytosol, and down regulates PCNA protein expression. In conclusion, we found that silibinin mediated silver nanoparticles triggered apoptosis in HepG2 cells. This activity was mainly due to ROS inducing potentials of silibinin mediated silver nanoparticles in HepG2cells. Accumulation of ROS is responsible for the morphological changes related to apoptosis and loss of MMP and increased caspase-3 expression.[2]

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References

(1)Ponselvi Induja M, Ezhilarasan D, Ashok Vardhan N. Evolvulus alsinoides methanolic extract triggers apoptosis in HepG2 cells. Avicenna J Phytomed. 2018;8(6):504-512.

(2)Ezhilarasan D, Evraerts J, Brice S, Buc-Calderon P, Karthikeyan S, Sokal E, Najimi M.Silibinin Inhibits Proliferation and Migration of Human Hepatic Stellate LX-2 Cells. J Clin Exp Hepatol. 2016;6(3):167-174.