SUMMARY

The need of novel and effective cancer drugs fascinates the researchers to continue the hunt and to explore more in the field of natural products. *G. lucidum*, growing in the environmentally stressed conditions was found to significantly enhance the synthesis of various bio-constituents, allowing it to sustain in the stress. From the host plant-*G. lucidum* relationship studies, it has been observed that the levels of active bio-constituents in *G. lucidum* are high when it grows on *Azadirachta,* as compared to other host plants. The total sugars, reducing sugars, phenols, starch, proteins, free radical scavenging potential, and flavonoids were found to be enhanced many folds. Also, this enhancement in the bio-constituents was several folds as compared to the mushrooms grown under normal environmental conditions. The crude extract of *G. lucidum* obtained from *Azadirachta* plant exhibits strong anticancer potential as compared to the *G. lucidum* grown on other host plants. GAs were the predominant terpenoids found in the aqueous methanolic extract of *G. lucidum*. Various isoform of GAs were explored for their binding interaction in the membrane receptors. Computational studies predicted the binding orientations of GAs with a number of different receptors. The binding of GAs with RTKs members exposed the various residues, binding interaction, and energy involved in the interaction. Protein-ligand interactions elucidated the strength of binding forces responsible for lipophilic, hydrogen bonding and π-π stacking during the molecular docking studies.

GAs were isolated from *G. lucidum* grown on the host plant *Azadirachta*. Isolated GAs potentially inhibited the cancer cells and showed 50% of inhibition at 50µg/mL and/or more concentration, after 48h treatment. Furthermore, GAs reduced the cell viability in lung, prostate, and breast cancer cells. These studies demonstrated the anti-proliferative potential of GAs in dose-dependent manner. GAs enhanced the rate of ROS production in DCFDA assay. On the other hand, GAs decreased the level of superoxide ion in cancer cells expressed through NBT assay. Thus, it can be concluded that the increase in ROS levels might be due to increased free radicals.

GAs suppressed the cell migration to the denuded zone in time-dependent manner. GAs were found more potent against the breast cancer cells as compared to lung and prostate cancer cells. Cancer cells represent a change in the mesenchymal shape

through EMT (Epithelial-to-Mesenchymal Transition) to mesenchymal expression. These systematic differences might be due to the active role of MMPs and other adhesive strengthening proteins which brings the migration of the cancer cells.

GAs reduced the colony formation and this phenomenon was more prominent in the prostate cancer as compared to breast and lung cancer cells. The current study demonstrated a concentration-dependent ROS production in the cancer cells treated with GAs. As analyzed by DCFDA assay, PC-3 and MDA-MB-231 cells showed enhanced production of ROS as compared to A549 cells. GAs arrest the cell cycle atG0/G1 phase in PC-3 cells and at G1 phase in A549 cells. Interestingly, no alteration in cell cycle was observed in MDA-MB-231 cells. Thus, GAs arrest cell cycle in different phases depending upon the type of cancer cells and control the functioning of the cell. GAs were found to reduce the mitochondrial membrane potential and induce DNA fragmentation, and nuclear fragmentation. This lead to alteration in the cell cycle and induce the process of apoptosis. GAs significantly decreased mitochondrial-mediated and induces the process of apoptosis. The enhanced level of ROS production caused induction in nuclear shrinkage, chromatin condensation, and nuclear fragmentation in a dose-dependent manner. GAs induced inter-nucleosomal DNA fragmentation which appeared more prominent in treated cells as compared to the control cells.

GAs target and suppress the expression of PI3K, Akt, and mTOR which activates and inducing the process of apoptosis. The effect of GAs treatment on the cancer cells and expression of different factors vary depending upon the nature of cell lines, their specification and response to various receptors present in them. GAs modulated the expression of bax, bcl-2, MMP-2, and MMP-9 which influence the process of apoptosis. GAs increased the expression of bax and decreased the expression of anti-apoptotic bcl-2 in A549 and PC-3 cells. Similarity, GAs up-regulated bax expression, but doesn't cause any change in bcl-2 expression in MDA-MB-231 cells. Decreased expression of MMP-2 and MMP-9 were found in cancer cells and the results were better for the breast cancer as compared to the lung cancer cells. The decreased level of MMPs, induces the process of apoptosis. Hence from the current

study, it can be concluded that GAs emerge as potential therapeutic modulators in the cancer signalling in the lung, prostate cancer, and breast cancer.

The results and leads obtained in the current studies can be utilized for future research in the similar area. GAs need to be purified and characterized by spectroscopic methods. These pure chemical constituents need to be screened for anticancer activities to find the most active constituent. The area of *G. lucidum* host plant association can be studied by chemical profiling of host-plant which changes with saprophytic nature of mushroom. Further, anticancer potential of various GAs can be explored in *in vivo* studies. Based on the molecular modelling studies some GAs have been identified which showed very good binding affinities at different receptors. Thus, different species of *G. lucidum* can be screened for the presence of these constituents and wet lab experiments (assays) can be performed to compare the results with computational studies. Further, GAs rich fractions could be subjected to column chromatography for the isolation and purification of individual component. Experiments can be performed to check whether the isolated pure component is responsible for the anticancer activity or it is a synergistic effect.