Chapter 6

Summary and Conclusion

6.1. Summary of results

- RSV exhibits preventive effect against several types of cancer such as breast, colon, gastric, pancreatic, prostate, lung, liver, thyroid, ovarian, muscle, head & neck cancer, leukemia etc. Recently, RSV has been proved for its anticancer potential against glioma. Though RSV showed strong efficacy against glioma, its therapeutic applications are limited because of short biological half life, lesser systemic circulation, rapid metabolism and elimination.

- In our research, we aimed to formulate RSV loaded TPGS and DSPE PEG 2000 coated solid lipid nanoparticles, PLGA:TPGS blend nanoparticles, core shell type polymer lipid hybrid nanoparticles and liposomes to prolong the systemic circulation and brain targeted delivery of RSV after intravenous (*i.v.*) administration in rats.

- As RSV is a lipophilic and poorly water soluble molecule (solubility = 30 mg/L; log P = 3.06), at first we formulated TPGS and DSPE PEG 2000 coated SLN formulations to prolong the systemic circulation and higher brain accumulation.

- RSV-TPGS-SLN and RSV-PEG-SLN showed the average particle size of 203.1±14.91 and 125.95±12.09 nm, respectively. Both SLN formulations showed narrow polydispersity index. Zeta potential of RSV-TPGS-SLN and RSV-PEG-SLN was found to be -10.5±2.94 and -24.43±3.27 mV, respectively. RSV-TPGS-SLN and

RSV-PEG-SLN showed the entrapment efficiency of 70.18±5.19 and 74.67±4.76%, respectively. Both SLN formulations showed sustained *in vitro* drug release up to 48 h. TEM studies revealed spherical shape of SLN formulations. FTIR, DSC and XRD studies suggest absence of potential chemical interaction and amorphous distribution of RSV in both SLN formulations.

- RSV-TPGS-SLN showed significantly higher cytotoxicity than RSV against C6 glioma cells ($p<0.05$) whereas RSV-PEG-SLN showed equal cytotoxicity to RSV ($p>0.05$). COU-TPGS-SLN and COU-PEG-SLN showed excellent cellular internalization in C6 glioma cells. Both the SLN formulations were found to be concentrated at cytoplasm of cancer cells which is the major site of action of RSV for anti cancer activity.

- RSV-TPGS-SLN and RSV-PEG-SLN were found to be haemocompatible in nature. Pharmacokinetic studies showed higher AUC, $t_{1/2}$ and MRT and lower CL of RSV-TPGS-SLN and RSV-PEG-SLN than that of RSV solution. These results strongly suggest that both SLN formulations will be the best suitable tool for improving systemic circulation of RSV. The brain distribution of RSV-TPGS-SLN and RSV-PEG-SLN was found to be 9.23 and 8.60 times higher than that of RSV solution, respectively, which indicates the passive brain targeting potential of SLN.

- In SLN formulations, though all pharmacokinetic parameters and brain accumulation are improved in comparison to RSV solution, the biological half life of RSV-TPGS-SLN and RSV-PEG-SLN were found to be only 5.53±0.55 and 8.22±1.36 h, respectively. The biological half life values indicate that multiple doses are required

to maintain the therapeutic concentration in plasma. The lesser biological half life may be due to RES uptake of SLN formulations due to its lipophilic nature. Polymer: surfactant blend nanoparticles are shown to have prolonged systemic circulations, higher biological half life and lower RES uptake. Therefore, we attempted PLGA: TPGS blend nanoparticles to further improve the biological half life.

- As the RSV loaded DSPE PEG 2000 coated nanoformulations were not stable, evaluations were performed only on TPGS coated BNPs. RSV-PLGA-BNPs showed lower particle size (175.5±8.47 nm), narrow polydispersity index and high entrapment efficiency (61.81±3.57%). Zeta potential of RSV-PLGA-BNPs was found to be -16.86±1.59 mV. RSV-PLGA-BNPs showed sustained drug release up to 48 h which is beneficial for prolonged action. The shape of RSV-PLGA-BNPs was found to be spherical. FTIR results revealed absence of potential chemical interaction between RSV and other nanoparticulate components. DSC and XRD studies revealed amorphous distribution of RSV in BNPs formulations.

- RSV-PLGA-BNPs showed significantly higher cytotoxicity in C6 glioma cells than that of RSV ($p<0.05$). The additive cytotoxic potential of RSV-PLGA-BNPs due to TPGS will be more beneficial in the treatment of glioma. COU-PLGA-BNPs showed excellent cellular internalization in C6 glioma cells and highly concentrated at cytoplasm.

- In pharmacokinetic studies, RSV-PLGA-BNPs showed higher AUC, $t_{1/2}$ and MRT and lower CL in comparison to RSV solution. Therefore, RSV-PLGA-BNPs will be an excellent alternative tool for improving half life as well as prolonged systemic

circulation for improved therapy. Brain distribution of RSV-PLGA-BNPs was found to be approximately 4.41 times higher than that of RSV solution.

- RSV-PLGA-BNPs showed the $t_{1/2}$ of 9.60±2.91 h whereas RSV-TPGS-SLN showed only 5.53±0.55 h ($p<0.05$). MRT of RSV-PLGA-BNPs (12.48±0.69 h) was found to be increased approximately twice in comparison to RSV-TPGS-SLN (6.84±0.23 h). Moreover, RSV was detected in blood up to 48 h when we administered RSV-PLGA-BNPs whereas in RSV-TPGS-SLN was present in blood only up to 36 h. Though few pharmacokinetic parameters were improved in RSV-PLGA-BNPs than that of RSV-TPGS-SLN, the brain distribution was significantly decreased in BNPs. Brain distribution of RSV-TPGS-SLN was found to be 7.48±1.69 µg/g whereas RSV-PLGA-BNPs showed only 3.57±0.53 µg/g. The higher lipophilic nature of SLN might be the reason for higher brain accumulation. BNPs favour prolonged systemic circulation and solid nanoparticles support higher brain accumulation.

- Core-shell polymer-lipid hybrid nanoparticles (HNPs) are novel drug delivery systems for high entrapment efficiency, sustained drug release, excellent serum stability, prolonged systemic circulation and brain targeting. Therefore, we aimed to investigate TPGS and DSPE PEG 2000 coated core-shell polymer-lipid hybrid nanoparticles to improve the biological half life, prolonged systemic circulation, higher brain accumulation and therapeutic efficacy of RSV.

- RSV-TPGS-HNPs formulation showed lower particle size of 145.5±9.7 nm, narrow polydispersity index of 0.217±0.11 and higher entrapment efficiency of 84.31±1.46%. RSV-PEG-HNPs prepared by DSPE PEG 2000 coating showed particle size of

238.4±12.6 nm, polydispersity index of 0.228±0.12 and entrapment efficiency of 76.59±2.49%. Zeta potential of optimized RSV-TPGS-HNPs and RSV-PEG-HNPs was found to be -7.63±0.29 and -15.46±2.34 mV, respectively. Both HNPs showed sustained drug release up to 48 h.

- FTIR and DSC results suggest absence of potential chemical interaction between RSV and other formulation excipients. XRD results indicated the conversion of RSV from crystalline to amorphous form in both HNPs. RSV-TPGS-HNPs showed higher cytotoxic potential in comparison to RSV-PEG-HNPs. Both HNPs showed excellent cellular internalization in C6 glioma cells and highly concentrated at cytoplasm. Pharmacokinetic studies showed higher AUC, $t_{1/2}$ and MRT and lower CL of both HNPs in comparison to RSV solution. Therefore, the proposed designs of RSV-TPGS-HNPs and RSV-PEG-HNPs will be excellent tools for prolonged systemic circulation. Brain distribution of RSV-TPGS-HNPs and RSV-PEG-HNPs was found to be 5.94 and 4.96 times higher than that of pristine RSV. Therefore, both HNPs can be applied as potential carrier to deliver drug across blood brain barrier by passive brain targeting potential.

- In terms of bulk scale up, the hybrid nanoparticles are not supportive for clinical applications. The brain distribution of both HNPs and BNPs was found to be lesser in comparison to SLNs. Several other lipid nanocarriers were proved for higher brain distribution in earlier investigations. Among them liposomes are the excellent carriers to deliver the drug across blood brain barrier as well prolonged systemic circulation.

Further, liposomal formulations are currently manufactured in large scale and widely marketed for chemotherapeutic applications.

- Liposomes are lipid bilayer vesicles proved for sustained delivery, prolonged systemic circulation and targeted delivery of chemotherapeutic drugs. In continuation of our previous attempts on solid lipid, polymeric and hybrid nanoformulaitons, TPGS and DSPE PEG 2000 coated and uncoated liposomes were attempted to improve the biological half life prolonged systemic circulation and passive brain accumulation of RSV.

- RSV loaded liposomal formulations were successfully prepared and evaluated for several liposomal characterizations. RSV-Lipo and RSV-TPGS-Lipo formulations showed mean vesicular size of 119.6±7.91 and 64.5±5.56 nm, respectively, with narrow polydispersity index. RSV-PEG-Lipo showed significantly higher particle size (250.2±9.03 nm) in comparison to uncoated and TPGS coated liposomes. RSV-Lipo, RSV-TPGS-Lipo and RSV-PEG-Lipo showed the zeta potential value of 3.47±0.97, -1.05±1.12 and -26.47±0.75 mV and entrapment efficiency of 82.45±3.72, 83.05±3.59 and 79.47±4.32%, respectively. All liposomes showed spherical shape in TEM micrographs.

- The liposomal formulations showed sustained RSV release up to 48 h. FTIR, DSC and XRD studies revealed absence of potential chemical interaction between RSV and other liposomal components.

- RSV-TPGS-Lipo showed significantly higher cytotoxicity in comparison to RSV, RSV-Lipo and RSV-PEG-Lipo in all concentrations against C6 glioma cells ($p<0.05$). The higher cytotoxic potential of RSV-TPGS-Lipo will be more beneficial in the treatment of glioma. COU-TPGS-Lipo and COU-PEG-Lipo showed excellent cellular internalization in C6 glioma cells. The liposomes were highly concentrated in the cytoplasm of the cells which is a major site of action of RSV for its anticancer activity.

- Haemolysis, erythrocyte membrane integrity and platelet aggregation studies revealed that both RSV-TPGS-Lipo and RSV-PEG-Lipo were of haemocompatible and safe for *i.v.* administrations. Pharmacokinetic results showed higher AUC, $t_{1/2}$ and MRT and lower CL of RSV-TPGS-Lipo and RSV-PEG-Lipo in comparison to pristine RSV and RSV-Lipo. RSV-TPGS-Lipo and RSV-PEG-Lipo showed 8.27 and 6.64 times higher brain distribution than that of pristine RSV. The higher $t_{1/2}$, higher brain distribution and lower liver distribution of RSV-TPGS-Lipo and RSV-PEG-Lipo supported that both liposomal designs will be the potential tool for prolonged systemic circulation as well as passive brain targeting.

Table 6.1. Summary of comparative results of all nanoformulations

Parameters	**RSV-TPGS-SLN**	**RSV-PEG-SLN**	**RSV-PLGA-BNPs**	**RSV-TPGS-HNPs**	**RSV-PEG-HNPs**	**RSV-TPGS-Lipo**	**RSV-PEG-Lipo**
Particle size (nm)	203.1±14.91	125.95±12.09	175.5±8.4	145.5±9.7	238.4±12.6	64.5±5.56	250.2±9.03
Polydispersity index	0.263±0.12	0.386±0.04	0.147±0.09	0.217±0.11	0.228±0.12	0.398±0.021	0.196±0.010
Zeta potential (mV)	-10.5±2.94	-24.43±3.27	-16.86±1.59	-7.63±0.29	-15.46±2.34	-1.05±1.12	-26.47±0.75
Entrapment efficiency (%)	70.18±5.19	74.67±4.76	61.81±3.57	84.31±1.46	76.59±2.49	83.05±3.59	79.47±4.32
Shape	Spherical						
Drug release	Sustained, Higuchi, anomalous						Higuchi, Fickian
Interaction /crystalinity analysis	No potential chemical interaction; RSV converted from crystalline to amorphous						
Cytotoxicity (in comparison to RSV)	Higher cytotoxicity	Similar cytotoxicity	Higher cytotoxicity	Higher cytotoxicity	Similar cytotoxicity	Higher cytotoxicity	Similar cytotoxicity
Cellular uptake	Excellent cellular internalization						
Haemocompatibility	Less than limit (10%), safe for *i.v.* administration						
Pharmacokinetic studies	Higher AUC, $t_{1/2}$ (5-8 h); present in blood up to 24 h		$t_{1/2}$ (9 h) Present in blood up to 36 h	Higher AUC, $t_{1/2}$ (10-15 h) present in blood up to 48 h)		Higher AUC, $t_{1/2}$ (11-15 h); present in blood up to 48 h	
Brain distribution (than RSV)	9.23 times higher	8.60 times higher	4.41 times higher	5.94 times higher	4.96 times higher	8.27 times higher	6.64 times higher

- Among the prepared nanoformulaitons, RSV-TPGS-Lipo shows lowest particle size of 64.5±5.56 nm, higher entrapment efficiency of 83.05±3.59%, higher $t_{1/2}$ of 15.72±2.96 h, lower clearance of 57.40±7.59 mL/h/kg, lower liver distribution and higher brain accumulation. Moreover, bulk manufacturing of liposomal formulations for clinical applications are also possible. Therefore, RSV-TPGS-Lipo is the best suitable formulation for chemotherapeutic application against brain cancer. RSV-TPGS-Lipo showed low zeta potential of -1.05±1.12 mV. The lower zeta potential may cause stability problems; however, it can be overcome by supplying the formulation as lyophilized powder.

6.2. Conclusion

RSV loaded TPGS and DSPE PEG 2000 coated solid lipid, polymeric, hybrid nanoparticles and liposomes were prepared and evaluated by various state of the art techniques. All nanoformulations showed optimum particles size, narrow polydispersity index and higher entrapment efficiency suitable for chemotherapeutic applications. No potential chemical interaction was found between RSV and formulation excipients. The nanoformulations were found to be haemocompatible and safe for *i.v.* administration. The prepared nanocarriers showed significantly higher AUC, $t_{1/2}$ and MRT in comparison to pristine RSV in pharmacokinetic study after *i.v.* administration. More over the brain accumulation of all nanocarriers was increased several folds than that of RSV solution. Therefore, the investigated design of SLN, BNPs, HNPs and liposomal formulations are suitable for prolonged systemic circulation and higher brain accumulation. Among the prepared nanoformulations, RSV-TPGS-Lipo is the best suitable formulation for chemotherapeutic application against brain cancer on account of lowest particle size (64.5±5.56 nm), higher entrapment efficiency (83.05±3.59%), higher AUC (30349.73±4850.28 ng*h/mL), maximum $t_{1/2}$ (15.72±2.96 h), lower clearance (57.40±7.59 mL/h/kg), lower liver distribution and highest brain accumulation. *In vivo* anticancer efficacy of the prepared nanoformulations is still need to be evaluated for potential clinical application against glioma.