**Abstract**

The present research is focused on the development of ecofriendly biopolymer blend based nanocomposites to enhance the effect of cytotoxic activity. Novel eco-friendly synthesis of pure Chitosan–Agar blend and Chitosan–Agar/ZnO nanocomposites was successfully synthesized by in-situ chemical synthesis method. The influence of Chitosan–Agar (1:1 wt/wt %) concentrations (0.1, 0.5, 1 and 3 g) was studied. The presence of ZnO nanoparticles in Chitosan–Agar polymer matrix was confirmed by UV, FTIR, XRD, FESEM, EDAX and TEM. The crystallite size of the nanocomposites in the range of 12–17 nm is observed from XRD analysis. PL and UV reveal that Nanocomposites shows an blue shift by increase in the blend concentrations. TEM analysis shows that 0.1 and 3 g of Chitosan–Agar/ZnO Nanocomposites are in spindle and spherical shape with polycrystalline nature. The prepared Nanocomposites shows the respectable Antibacterial activity against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Pseudomonas aureginosa* and *Klebsilla pneumonia*) bacteria. The potential toxicity of Chitosan–Agar/ZnO nanocomposites was studied for normal (L929) and breast cancer cell line (MB231). The result of this investigation shows that the Chitosan–Agar/ZnO nanocomposites deliver a dose dependent toxicity in normal and cancer cell line.