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Summary and Conclusion

Nanotechnology an emerging field of nanoscience is escalating day by day which deals with particles in the nanoscale due to its evocative physical and chemical properties rather than their bulk counterparts. The recent focus and application of nanotechnology towards medicine involves diagnosis, therapeutic and prevention against various diseases. Synthesis of nanoparticles by means of nature-based system has drawn attention, as they are eco-friendly. Among all the nanoparticles metallic nanoparticles such as silver, gold and iron have gained importance due to its wide bioactive properties.

Synthesis of metal nanoparticles from plant extracts and bioactive components present in it has haggard consideration recently, because of its economical and non-toxic nature. Moreover, functionalization of nanoparticles on their surface by means of a polymer provides stabilization to the nanoparticles from preventing its aggregation and control the oxidation of the nanoparticles.

The present research focused on **"Antioxidative and Antitumorigenic Potential of PEG Functionalized Silver Nanoparticles from Ethanolic Extract of *Volkameria inermis* leaves to EAC Cells by *in vitro* and *in vivo* Studies"** was carried out in five phases. The method of synthesis was optimized and the drugability, biocompatibility, antioxidative and anticancer properties of the synthesized PEG functionalized silver nanoparticles were evaluated.

In Phase I screening of bioactive constituents and scavenging of DPPH, superoxide, hydroxyl, hydrogen peroxide, nitric oxide and Ferric Reducing Antioxidant Power (FRAP) assay by the different extracts namely petroleum ether, chloroform, ethyl acetate, ethanol and water were carried out. The ethanolic extract of *Volkameria inermis* leaves showed maximum number of phytoconstituents and more radical scavenging activity than that of the other extracts. Hence, the ethanolic extract of *Volkameria inermis* leaves was chosen for further studies.The presence of quercetin and quercetin derivatives

in the ethanolic extract of *Volkameria inermis* leaves was confirmed by HPLC and HPTLC chromatogram.

In the second phase of the study three different methods namely microwave heating, heating in waterbath at 60°C and exposure to bright sunlight were adapted for the synthesis of silver nanoparticles. The synthesis of silver nanoparticles was monitored by a change in colour (qualitatively) and an increase in yield (quantitatively). Among the three different methods, sunlight exposure showed a notable colour change and yield from 5 to 20 minutes and selected for the rapid synthesis of silver nanoparticles from the ethanolic extract of *Volkameria inermis* leaves. The silver nanoparticles synthesized from the ethanolic extract of *Volkameria inermis* leaves was functionalized using PEG - 4000. The synthesized silver nanoparticles and PEG functionalized (PEGylated) silver nanoparticles were characterized by various techniques such as spectral analysis, Transmission electron microscope (TEM), Energy dispersive absorption spectroscopy (EDX), X- ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Zeta potential.

The UV – visible absorption spectra, showed the characteristic peak for silver nanoparticles at 430 nm. PEGylated silver nanoparticles gave a slight shifting of peak at 450 nm, due to the surface interaction of PEG with silver.

The TEM images of both the silver nanoparticles and PEGylated silver nanoparticles showed spherical shape, with a size of 10-18 nm and 25-35 nm respectively. All the nanoparticles were well dispersed without aggregation and may be ideal for biomedical applications.

The EDX and XRD profiles of both the nanoparticles showed the presence of typical peak for silver and also additional peaks which may be due to the presence of other phytoconstituents in the synthesized silver nanoparticles. FTIR spectra confirmed the presence of hydroxyl, alkene and aromatic groups which are involved in the synthesis of silver nanoparticles from the extract. An additional peak for C-OH in PEGylated silver nanoparticles showed the successful grafting of PEG on silver.

The zeta potential values for the synthesized silver nanoparticles and PEGylated silver nanoparticles were found to be 18.5 and -25.0 mV respectively. These values were

found to be well within the stable range (-30 to + 30 mV). The positively charged silver nanoparticles bind to the negatively charged oxygen of hydroxyl group present in PEG and confirmed the efficacious grafting of PEG on silver.

For any drug to be used for mankind the biocompatible nature of the drug plays a key role. Hence, the biocompatible nature of the PEG functionalized silver nano particles towards human red blood cells and the drug releasing profile at different pH conditions such as 7.4, 6.8 and 5.5 were studied in the third phase. The toxicity of PEGylated silver nanoparticles was tested on red blood cells of healthy human volunteers, to determine their biocompatibility. The results showed that the PEGylated silver nanoparticles did not cause hemolysis and have not changed the morphology of RBCs and proved to be a biocompatible agent and safety use in humans. The drug release profile of PEGylated silver nanoparticles showed a pH sensitive drug release behavior in all the tested pH conditions. At tumor pH 6.8 the drug release was found to be significant than the other pH conditions studied indicating the therapeutic efficacy towards tumor cells.

Having assessed the biocompatibility nature and drug releasing profile of PEGylated silver nanoparticles, the *in vitro* cytotoxic and apoptotic activities of ethanolic extract of *Volkameria inermis* leaves and PEGylated silver nanoparticles were carried out using EAC cells in the fourth phase. The cytotoxic effect of ethanolic extract of *Volkameria inermis* leaves and PEGylated silver nanoparticles was evaluated by MTT assay using EAC cells. Both the ethanolic extract of *Volkameria inermis* leaves and PEGylated silver nanoparticles exposed to EAC cell lines and intraperitoneally propagated EAC cells exhibited antitumorigenic effect in a dose dependent manner. Knowing the cytotoxic effect of ethanolic extract of *Volkameria inermis* leaves and PEGylated silver nanoparticles, the role of apoptotic effect was assessed by AO/EtBr dual staining, DNA damage by DNA laddering in agarose gel electrophoresis and cell cycle analysis using fluorescent activated cell sorting (FACS) assay. AO/EtBr staining revealed that the type of cell death observed was predominantly apoptosis. DNA laddering due to DNA fragmentation was found to be more pronounced in the PEGylated silver nanoparticles than that found in the ethanolic extract of *Volkameria inermis* leaves. Cell cycle analysis of PEGylated silver nanoparticles treated with EAC cells gave higher

(30.87 per cent) early apoptotic cells than the ethanolic extract (24.94 per cent) of *Volkameria inermis* leaves at G2 / M phase.

In the fifth phase antitumorigenic potential of nanoparticles was evaluated by *in vivo* study carried out in Swiss albino mice induced with intraperitoneally propagated EAC cells. The antitumorigenic activity was evaluated by assessing the mortality rate; liver marker enzymes; enzymic and non enzymic antioxidants; lipid peroxidation and histological status of liver of all the experiment groups compared to the standard antioxidant silymarin after 15 days and 60 days of the experimental tenure in 9 groups of six mice in each (Group 1- PBS; Group 2- DMSO; Group 3- Paraffin oil; Group 4 – Silymarin ; Group 5 – Ethanolic extract ; Group 6- PEGylated AgNPs ; Group 7 – EAC ; Group 8 - Ethanolic extract +EAC ; Group 9 – PEGylated AgNPs + EAC).

To determine the optimal dose for the *in vivo* antitumorigenic effect of PEGylated AgNPs, the *in vitro* cytotoxicity study was carried out by trypan blue exclusion assay. The PEGylated AgNPs showed a dose dependent cytotoxic effect to EAC cells. The EC_{50} was calculated (24µg/ml) and used for the *in vivo* studies.

The average life span of EAC cells bearing mice was 19 days. Administration of ethanolic leaves extract and PEGylated silver nanoparticles to EAC transplanted mice the average life span was found to be increased to 60 days indicating their antitumorigenic effect.

To assess the normal functioning of liver the liver marker enzymes namely aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were analysed in all the experimental animals. The activities of all the three liver marker enzymes were found to be significantly increased in EAC cells induced mice. The increase in the activities of these liver marker enzymes in the serum was due to hepatocellular damage by the unusual generation of free radicals in tumor burden leading to the leakage of cytosolic enzymes into the circulation. The administration of ethanolic leaves extract and PEGylated silver nanoparticles caused a significant decrease in the levels of liver marker enzymes. Coadministration of ethanolic leaves extract and PEGylated silver nanoparticles to EAC cells induced mice showed a significant decrease in the activities of AST, ALT and ALP. The above results showed the significant

protective effect of ethanolic leaves extract and PEGylated silver nanoparticles to maintain the normal functioning of the liver.

Intraperitoneal administration of EAC cells to Swiss albino mice altered the antioxidant balance of the mice liver by decreasing the activities of enzymic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and the levels of non enzymic antioxidants namely Vitamin A, Vitamin E and reduced glutathione (GSH). Administration of ethanolic leaves extract and PEGylated silver nanoparticles significantly enhanced the enzymic and non enzymic antioxidant status in both the treatment periods. This enhancement was found to be more significant than that of silymarin administered group. Enzymic and non enzymic antioxidants protect the cells by quenching the free radicals generated by EAC cells. The above results revealed the effective enhancement of antioxidative potential of PEGylated silver nanoparticles when compared to the ethanolic extract of *Volkameria inermis* leaves.

The levels of malondialdehyde (MDA) the end product of lipid peroxidation were found to be significantly increased in the liver of EAC cells transplanted mice. The administration of ethanolic leaves extract, PEGylated silver nanoparticles and silymarin caused a significant decrease in the levels of MDA. Coadministration of ethanolic leaves extract and PEGylated silver nanoparticles to EAC cells induced mice caused a significant decrease in the levels of MDA. Thus, the observations of the study proved the antilipid peroxidative role of ethanolic leaves extract, PEGylated silver nanoparticles and silymarin. Ethanolic extract of *Volkameria inermis*, PEGylated silver nanoparticles and silymarin strengthened the endogenous antioxidant defense by scavenging the reactive oxygen species and maintained the optimal balance of free radicals and antioxidative status of the tissues.

The histological observations of all the control and drug treated groups revealed normal architecture in their portal triads, sinusoids, Kupffer cells and central veins. The histological observations of EAC cells treated mice showed necrosis with focal statosis and balloon degeneration. The liver of ethanolic leaves extract and PEGylated silver nanoparticles administered to EAC cells induced mice showed normal architecture. The investigations of the *in vivo* study evidently exposed the antioxidative and

antitumorogenic role of silver nanoparticles. The findings of the present study, thus validated the method for the synthesis of AgNPs and PEGylated AgNPs from the ethanolic extract of *Volkameria inermis* leaves and their biomedical applications as drug carriers for targeted drug delivery system to EAC cells induced mice. PEGylated AgNPs was found to be more efficient antitumorigenic agent than its corresponding ethanolic extract of *Volkameria inermis* leaves.

Conclusion

To conclude **this was the first report** using PEGylated silver nanoparticles of *Volkameria inermis* as drug delivery system to deliver the PEG functionalized silver nanoparticles as an antitumorigenic agent against EAC cells induced Swiss albino mice, which was evident by their *in vitro* and *in vivo* antioxidative and antitumorigenic potential. Hence, the PEG functionalized silver nanoparticles can be recommended as an antioxidant and antitumorigenic agent to overcome the oxidative stress in individuals suffering from oxidative degenerative diseases including cancer.

Suggestions for future research

The outcome of the present research work has opened up a number of boulevards for further research. The works that can be suggested for future research are given below.

- Metallic nanoparticles other than silver such as gold and iron can be synthesized from the individual phytoconstituent of ethanolic extract of *Volkameria inermis* leaves and its anticancer property can be studied.
- *In silico* docking analysis can be carried out to study the interaction of PEG functionalized silver nanoparticles with the biological target molecules.
- The proteomic and genomic profiles of cancer cells treated with PEG functionalized silver nanoparticles can be scrutinized to understand the mechanism of anticancer effect at the protein and gene levels.