

Introduction

Medicinal plants are one of the fascinating nature's gift which play an important role as a raw material for drugs, due to the presence of active phytochemicals such as alkaloids, flavonoids, tannins, carotenoids and primary metabolites such as glycosides, proteoglycans and glycoproteins which help in preventing various oxidative stress associated diseases. The therapeutic potential of medicinal plants as antioxidants cause protective effect by neutralizing free radicals, the toxic byproducts of cell metabolism (Wojtowicz *et al.*, 2017; Santhi and Sengottuvel, 2016; Akhtar *et al.*, 2015). The use of medicinal plants was proven to be efficacious, safe, less toxic, accessible and reliable natural sources.

Free radicals are molecules with an unpaired electron due to which they are highly unstable, trying to capture electrons from nearby molecules and cause deterioration of cells (Bhandari *et al.*, 2017). This chain reaction continues and causes damages to DNA, oxidations of polyunsaturated fatty acids in lipids, oxidations of amino acids in proteins and oxidatively inactivate specific enzymes by oxidation of co-factors (Cui *et al.*, 2012).

Phytoantioxidants combat the free radicals formed and terminate the chain reaction before vital organs are damaged (Sivaraj *et al.*, 2018). There is a growing need all over the world in using medicinal plants as a source of antioxidants. The antioxidant role of medicinal plants can suppress the oxidative stress associated diseases. The toxic side effects of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) can be overcome by the natural antioxidants derived from plants. Hence, researchers have focused their interest on plant derived natural antioxidants (Narayanaswamy and Balakrishnan, 2011). The biological activity of the phytoactive compounds might vary depending upon the nature of the bioactive constituents which can be tackled by the synthesis of nanoparticles which improve the bioavailability, absorption and potential biological activity (Gupta and Birdi, 2017).

Cancer is a term used for a fatal disease in which abnormal cells divide without control and invades other tissues. It spreads from one part of the body to the other through blood and lymph systems (US National Cancer Institute, 2014). In 2015, the incidence of cancer was found to be 700,000 (WHO). In 2030, about 21.7 million new cases are expected to be diagnosed and 13 million cancer deaths are expected to be recorded (www.cancer.org; Estanqueiro *et al.*, 2015). The death rate of cancer was found to be higher in persons whose lifestyle practices includes alcohol consumption, smoking, lack of physical activity and obesity. Moreover, genetic factor also accounts for increase in cancer death rate (Siegel *et al.*, 2015).

Cancer can affect the normal cells and the process of disease differs due to various factors such as alcohol, tobacco, diet, occupation, pollution, reproductive and sexual behavior. The standard treatments followed for cancer are surgery, radiotherapy, and chemotherapy. These treatment modalities are costly, mutilating and has serious side effects, associated with residual morbidity (Wang *et al.*, 2018; Coccia and Wang, 2015). However, various research efforts worldwide results several promising strategy for cancer prevention.

In this context, nanooncology an emerging field of nanoscience is running as a precursor for cancer therapy. The conventional chemotherapeutic drugs which are highly insoluble and impermeable can be overcome by nanomedicine (Wang *et al.*, 2015). In the last few decades about 85,000 compounds have been screened against cancer, but only few of them are considered as effective anti-cancer drugs (Kura *et al.*, 2014). To overcome these disadvantages nano drug based therapy creates a new path for the development of novel therapeutic approaches in cancer management.

Nanotechnology a promising field of nanoscience involves the creation, manipulation and application of nano sized particles in the field of nanomedicine. It deals with particles having a size of 1-100 nm. These nanomaterials are considered as “intelligent” or “smart” materials used for constructing nanocarriers, which plays a vital role in controlled drug delivery systems and possess high biocompatibility nature (Shi *et al.*, 2014). Nanoparticles have received special attention due to greater surface area to volume ratio and highly reactive than macromolecules. Employing nanotechnology, green method for the synthesis of nanoparticles provides tremendous potential to fight against cancer (Ahmed *et al.*, 2016; Gnanajobitha *et al.*, 2012).

Among the various metal nanoparticles synthesized (such as silver, gold, iron, zinc and platinum), silver nanoparticles have gained more importance in the nanotechnology field. As, silver in the nano size is safe inorganic and non-toxic agents and encompasses a wide range of applications such as antibacterial and antifungal effects (Ashokkumar *et al.*, 2015).

Recently, much research work has been carried out in the development of green synthesis method of nanoparticles. More than 200 plants belonging to different families were screened for their ability to synthesis gold, iron, silver, copper and palladium by means of green synthesis approach in the field of nanomedicine. The synthesis of silver nanoparticles from plant not only facilitates the effective physicochemical properties, but also carries potential biological properties like antimicrobial and anticancer activity (Rajan *et al.*, 2015; Rauwel *et al.*, 2015). A research conducted by Devi and Bhima (2012) showed that the silver nanoparticles synthesized from *Ulva lactuca* showed potent anticancer activity against laryngeal carcinoma (Hep2), breast cancer (MCF-7) and colorectal cancer cells (HT29). Similarly, Kalaiarasi *et al.* (2015) reported that the silver nanoparticles synthesized from bamboo leaf extract resulted higher cytotoxic effect on human prostate cancer cells (PC3). In another research, silver nanoparticles synthesized from *Eucalyptus chapmaniana* leaf extract showed effective anticancer activity against HL-60 cell line (Sulaiman *et al.*, 2013).

Employing plant and plant-based materials for the biosynthesis of nanoparticles, technically called as green technology does not involve any harmful chemicals (Paulkumar *et al.*, 2017; Gopinath *et al.*, 2013). The production of silver nanoparticles and its yield depends on the nature of the plant extract, its concentration, concentration of the metal salt, temperature and contact time are recognized as necessary factors (Rossi *et al.*, 2014).

Silver nanoparticles synthesized from plant extract have resulted that silver capped with the functional groups of active phytoconstituents of the extract, acts as antioxidant agents and enhance the biological activity like anticancer effect (Khan *et al.*, 2017; Hussien and Siddiqi, 2014). It has a great potential in cancer management, they selectively disrupts the mitochondrial respiratory chain leading to the production of reactive oxygen species (ROS), induces the expression of genes associated with DNA

damage and enhanced apoptosis of tumor cells. It induces cytotoxic effects to cancer cell in a dose and size dependent manner.

Functionalization of silver nanoparticles during its synthesis by means of a stabilizer plays a great role in the arrangement and scattering strength of metal nanoparticles. For this reason, polymers have been broadly utilized as a molecule stabilizer to control the nanoparticle aggregation, settle the metal dispersion and control the oxidation of the particle. Polyethylene glycol (PEG) is a water soluble polymer used as a good stabilizer for silver nanoparticles. It is used to control the formation and dispersion stability of metal nanoparticles (Sun *et al.*, 2001; Pimpang *et al.*, 2008). Generally, PEG coated nanoparticles when dispersed reduces the adsorption of non-specific protein and clearance by macrophages, which facilitates the nanoparticles to cross the cell membrane (Hoa *et al.*, 2009; Zhang *et al.*, 2002).

For an effective delivery of a drug, the drug delivery systems need to penetrate into the biological system where the effective therapeutic compound exerts its pharmacological effect. Nanodrug delivery system provides a great platform to achieve the drug system effectively by targeted drug therapy for various diseases. However, the unique property of the nanoparticles, increased surface area to volume ratio increase the burst release of the drug, where the drug release rate depends on the rate of diffusion and the stability within the delivery system. Sustained drug release increases the therapeutic efficacy and decreases the side effects (Alaqad and Saleh, 2016; Loira – Pastoriza *et al.*, 2014).

Various treatments such as chemotherapeutic and radiotherapeutic options are available for cancer. But, the critical challenge is to deliver these drugs specifically to cancer cells with no tremendous toxic effects to normal cells (Thangam *et al.*, 2015). Drug delivery systems by means of nanoparticles have shown increased efficacy in cancer treatment due to their improved pharmacokinetics and biodistribution profiles by means of enhanced permeability and retention (EPR) effect (Du *et al.*, 2011). The EPR effect enhances the bioavailability of the drug by stimulated internalization of nanoparticles and sufficient drug release within tumor tissues (Zhu *et al.*, 2005). Among the stimuli pH responsiveness is the most frequently investigated, since pH values differ

appreciably in different tissues and cellular compartments such as in tumor cells pH 6.8; in blood and normal tissues pH 7.4 and late endosome and lysosome pH 5.0-5.5 (Chiang *et al.*, 2012; Sun *et al.*, 2010; Wang and Langer, 2012; Hu *et al.*, 2013). Our present research work was aimed to design a potentially capable pH responsive PEG functionalized silver nanoparticles drug delivery system specific to EAC cells.

Volkameria inermis (L.) is an evergreen sprawling shrub 1-1.8m tall. It is widely found in the tropical regions of India, Nepal, Bangladesh, Sri Lanka, Southeast Asia and Mediterranean (Rabiul *et al.*, 2011). The stems are woody and smooth. Leaves are found to be simple, ovate and elliptically long (5-10 cm), the upper two surface of the leaves are slightly shiny with entire margins, oppositely arranged. The cyme is comprised of three flowers joined at a common base point, white corolla and fused with five lobes. The stamens are four in number, reddish to purple in colour which is upwardly curved. The fruit is 1-1.5 cm long green in colour which turns black (Turner and Wasson, 1997; Ling 2001). Its synonym is *Clerodendrum inerme* and it is commonly known as Wild Jasmine, Seaside Clerodendrum, Sorcerers Bush, Indian privet, Garden quinine and Embret, in Tamil it is known as Sangam. The Taxonomic classification of the plant is: Kingdom: Plantae, Subkingdom: Tracheobionta, Superdivision: Spermatophyta, Division: Magnoliophyta, Class: Magnoliopsida, Subclass: Asteridae, Order: Lamiales, Family: Verbenaceae, Genus: *Volkameria* L., Species: *Volkameria inermis* (L.) Gaertn. Traditionally the roots and leaves of this plant were used as a febrifugal, uterine stimulant, a pest control agent and antiseptic, to arrest bleeding, treatment of asthma, hepatitis, ringworm and stomach pains (Muthu *et al.*, 2006). The plant was enriched with bioactive constituents such as cardiac glycosides, anthraquinones, proteins, phenolics, flavonoids, saponins, tannins, iridoids, diterpenes, triterpenes, sterols, steroids, carbohydrates, fixed oils, volatile oils and lignin. It exerts many pharmacological effects such as anti-inflammatory, analgesic, antipyretic, neural and smooth muscle effects, antimicrobial, antidiabetic, antioxidant, antiparasitic, insecticidal, antiallergic, anticancer, protective and many other pharmacological effects (Chethana *et al.*, 2013).

Till date there is no research on **Antioxidative and Antitumorigenic Potential of PEG Functionalized Silver Nanoparticles from Ethanolic Extract of *Volkameria***

***inermis* Leaves to EAC Cells.** In order to provide scientific validation the study was carried out with the following hypothesis and objectives:

Hypothesis

The study 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 laid down by the following hypothesis.

The **null hypothesis (H₀)** framed for our study was:

The green synthesized PEGylated silver nanoparticles from *Volkameria inermis* leaves, do not show good anticancer effect.

The **alternate hypothesis (H_A)** formulated was:

The green synthesized PEGylated silver nanoparticles from *Volkameria inermis* leaves, have good anticancer effect.

Objectives

To test the above hypotheses, the study was carried out in five phases with the following objectives:

- To screen the phytochemicals and evaluate the antioxidative role of different extracts of *Volkameria inermis* leaves
- To optimize the method for the synthesis of silver nanoparticles, synthesis of PEG functionalized silver nanoparticles and their characterization
- To determine the biocompatibility and the drug release profile of PEGylated silver nanoparticles
- To detect the *in vitro* cytotoxic and apoptotic effect of PEGylated silver nanoparticles using EAC cells
- To evaluate the *in vivo* antioxidative and antitumorigenic potential of PEGylated silver nanoparticles using intraperitoneally propagated EAC cells in Swiss albino mice.