Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2018; 10(7): 176-182

ISSN- 0975 1556

Research Article

Paclitaxel Loaded Magnetic Nanoparticles Induce *In vivo* Tumor Growth Inhibition in a Rat Glioblastoma Model

Ramulu Chintala[1], Fahima Dilnawaz[2], Manas Panigrahi[3], Sanjeeb K Sahoo[2], Phanithi Prakash Babu[1*]

[1]Department of Biotechnology and Bioinformatics, School of Life Sciences, University of Hyderabad, Hyderabad-500046. India
[2]Laboratory of Nano-medicine, Institute of Life Sciences, Bhubaneswar-751023. India
[3]Department of Neurosurgery, Krishna Institute of Medical Science, Secunderabad -500003. India

Available Online: 25th July, 2018

ABSTRACT
Glioblastoma multiforme (GBM) is one of the most common and lethal diseases and the current therapeutic options remain sparse. The magnetic nanoparticles (MNPs) based drug delivery approach for the cancer treatment has become one of the promising options to achieve controlled drug delivery to target tumor. In the present study the efficacy of paclitaxel loaded glyceryl monooleate (PAC-GMO)-MNPs was evaluated by administering it in a glioblastoma induced rat model. The results of H&E and immunohistochemistry (IHC) suggest that the treatment with PAC-GMO-MNPs significantly reduced or inhibit the tumor growth as compared to the native drug and may be the potential vehicle for efficiently treating GBM.

Keywords: Glioma, paclitaxel, Magnetic nano particles.

INTRODUCTION

GBM is one of the most aggressive type of primary brain tumors with a low survival rate[1,2]. However, effective treatment is so far not available due to complex nature of the tumor environment. The presence of Blood brain barrier (BBB) limits the effective entry of a majority of chemotherapeutic drugs, and thus severely restricts therapy for glioma. Moreover, these tumor cells are resistant to chemotherapeutic drugs largely due to multidrug resistance (MDR). Conventional methods are applied to deliver different drug molecules to the brain tissues, such as osmotic disruption of the BBB, intraventricular drug injection or local therapy/implants[3-5]. Nanotechnology based drug delivery approach is one of the upcoming approach for the treatment of brain tumor and neurodegenerative diseases, as the particulate system can cross the BBB with adsorption on the capillary walls, opening of the tight junctions and transcytosis[4,6]. Therefore, nanocarriers with appropriate surface functionalization, can be effectively used to transport various kinds of molecules across the BBB[7,8]. Nanoparticulate drug delivery to the brain preferably depends upon the coating of various polysorbates, as it can adsorb the apolipoprotein E from the blood stream onto the nanoparticle surface, and in turn mimic as Low density lipoprotein (LDL) particles, that interacts with the receptors of LDL leading to the uptake by endothelial lining of the BBB[4]. Many polysorbates as coating materials have been able to cross BBB and are evaluated for the efficacy of brain targeting, out of which polysorbate 80 illustrates better brain targeting effect[4]. MNPs based drug delivery approach offers the potential treatment due to the biocompatiblilty, biodegradability, non-toxicity, and the high-level accumulation in the target tissue or organ[9]. The other advantage in case of MNPs for brain delivery is that significant amount of doses can be accumulated at the tumor site with the aid of an external magnet[10]. Exploiting this advantage, many investigators have shown vivid examples of drug targeting to its specific sites through magnetic material[11,12]. Paclitaxel is a chemotherapeutic drug for the treatment of wide range of cancers including brain tumor. However, it is unable to traverse the BBB and attain a therapeutic dose in brain tissues as it is associated with efflux transporters p-glycoprotein (P-gp)[13]. Recently, researchers adapted diverse approaches for targeting brain tumors as these tumors utilize enormous amount of natural fatty acids for their growth and energy. Therefore, tumors were targeted with natural fatty acid and its synthetic derivatives[14]. Using prodrug approach, Ke et. al, conjugated linoleic acid (CLA) to paclitaxel which showed higher anti tumor activity in brain tumor bearing rats than that of native paclitaxel[15]. In our previous study we have reported, different hydrophobic and hydrophilic drugs can be entrapped inside the polymeric cages GMO-MNPs and its potential in drug delivery[16,17]. In another study, we have also demonstrated significant amount of paclitaxel loaded GMO-MNPs can be transported to the brain tissues as compared to native paclitaxel in healthy rat under physiologic condition[18]. In the present study GMO coated MNPs are very small in size and have the potential ability

**Author for Correspondence:*

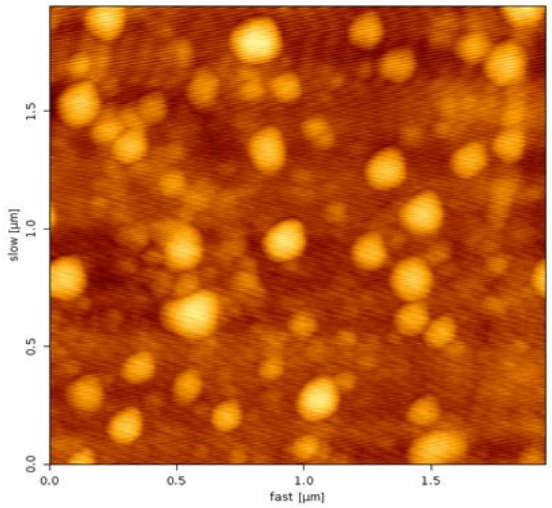

Figure-1. Representative image of surface topology of PAC-GMO-MNPs by Atomic Force Microscopy.

to accumulate inside the tumor tissues by crossing the BBB, and hence the therapeutic efficacy of the paclitaxel loaded GMO-MNPs was evaluated for GBM after systemic administration in a rat glioma model.

MATERIALS AND METHODS

Materials

Iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$) 99%, Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) pure granulated, 99%, ammonium hydroxide, 3-aminopropyl triethoxysilane (saline) were purchased from (Sigma Aldrich, St Louis, MO, USA), and acetonitrile of HPLC grade was procured from E-Merck, India. Alcohol, Xylene, Acetone and other chemicals, inorganic salts used were of analytical grade from (SRL chemicals and Qualigens Fine Chemicals, Mumbai, India). Glyceryl monooleate (GMO) was obtained from Eastman Chemical Company (Memphis, TN, USA). Paclitaxel was purchased from Shaanxi Schiphar Biotech Pvt Ltd, China. Milli Q water purged with nitrogen (N_2) gas was used in all steps involved in the synthesis and formulation of magnetic nanoparticles. Stereotaxic apparatus and infusion pump purchased from Stoelting Co. IL, USA.

Preparation of drug loaded magnetic nanoparticles

The preparation of PAC-GMO-MNPs were done following our previous published protocol [16]. In brief, 0.1 M Fe (III) dissolved in 50 ml N_2 purged water and 0.1 M Fe (II) dissolved in 50 ml N_2 purged water from which, 15 ml of Fe (III) and 7.5 ml of Fe (II) were mixed and heated at 80°C for 10 min under constant stirring with a magnetic stirrer in N_2 atmosphere. To that 1.5 ml of ammonium hydroxide (14.5 M) was added drop wise and thereafter, to that, 100 % w/w GMO was added and stirred for 20 min in N_2 atmosphere. Finally the precipitate was cooled to room temperature and then washed with ethyl acetate and acetone in the ratio of 70:30 (v/v) twice and finally with N_2 purged water, at 20,000 rpm for 20 min at 10°C (Sigma centrifuge, 3-16PK, Osterode, Germany). The obtained pellets dispersed in Milli Q water, was frozen at – 80 °C and lyophilized with a lyophilizer (LABCONCO Corporation, USA) for two days. For loading of the drug, 100 mg of MNPs particles were dispersed in 10 ml of nitrogen purged Milli Q water, sonicated (VC505, Vibracell, Sonics, Newton, USA) for 1 min at 30 % amplitude and left for stirring. Thereafter, 10 mg paclitaxel (dissolved in ~1 ml acetonitrile) was added drop wise to the dispersion and left for overnight stirring. Next day, the particles were washed with N_2 purged Milli Q water for 3 times at 20,000 rpm for 20 min at 10°C (Sigma centrifuge, 3-16PK, Osterode, Germany) and the pellets were lyophilized as stated above and the obtained lyophilized powder was used for further experimental studies.

Physicochemical characterization

The three dimensional surface topology of the paclitaxel loaded magnetic nanoparticles **(**PAC-GMO-MNPs) was observed by Atomic Force Microscopy (AFM) (JPK Nanowizard II, JPK instrument, Bouchestrasse, Berlin, Germany). For this, a drop of PAC-GMO-MNPs solution in distilled water (~ 1mg/ml) was dropped over freshly cleaved mica and left for 5 min, air dried at room temperature and mounted on microscope scanner and imaged on a contact mode having a frequency of 13 kHz and scan speed of 1 Hz. The amounts of encapsulated paclitaxel in MNPs were estimated by the reverse phase isocratic mode of high performance liquid chromatography (RP-HPLC) as reported by us previously[16]. Briefly, lyophilized PAC-GMO-MNPs was dissolved in acetonitrile (~1 mg/ml), sonicated for 1 min in an ice bath and kept in the shaker at 15°C and 150 rpm (Innova 43 Newbrunswick Scientific, Eppendorf, Germany) for 24 h for proper dissolution of the particulate formulations and complete release of the entrapped drug. After that, the samples were removed and centrifuged at 13,800 rpm for 10 min at 10°C (Sigma centrifuge, 1–15 K, Germany). The supernatant was collected and from this 500 µl was taken out for analysis using (WATERS™ 600 RP-HPLC system. 20 µl of supernatant was injected manually in the injection port, quantified at a flow rate of 1 ml/min with mobile phase of acetonitrile: water, (80: 20 v/v) with a quaternary pump (M 600E) at 25 °C, using C18 column (Nova-Pak, 150 x 4.6 mm, internal diameter) at 228 nm using UV-VIS absorbance detector (M2489). The standard curve of paclitaxel was prepared under identical conditions and the entrapment efficiency was calculated as reported earlier[16].

Cell culture

C6 rat glioma cell lines were procured from National Centre for Cell Science (NCCS), Pune, India. Cells were grown in RPMI media (Gibco, Invitrogen Corp) containing 10% FBS (Invitrogen Corp), 1 % antibiotics i.e., penicillin G (Gibco, Invitrogen Corp) and 100 mg/ml streptomycin (Gibco, Invitrogen Corp) and maintained in CO_2 incubator at 5% CO_2 (MCO-19M. Sanyo). Healthy 80 % confluent C6 cells were trypsinized and pellet collected, cell count done with haemocytometer.

Development of rat glioblastoma model and treatment

Male Wistar rats (3 months) were procured from Mahaveer agencies, Hyderabad, India. Rats were housed for one week for acclimatization prior to stereotaxic surgery. Rats were deeply anesthetized with ketamine and xylazine (100

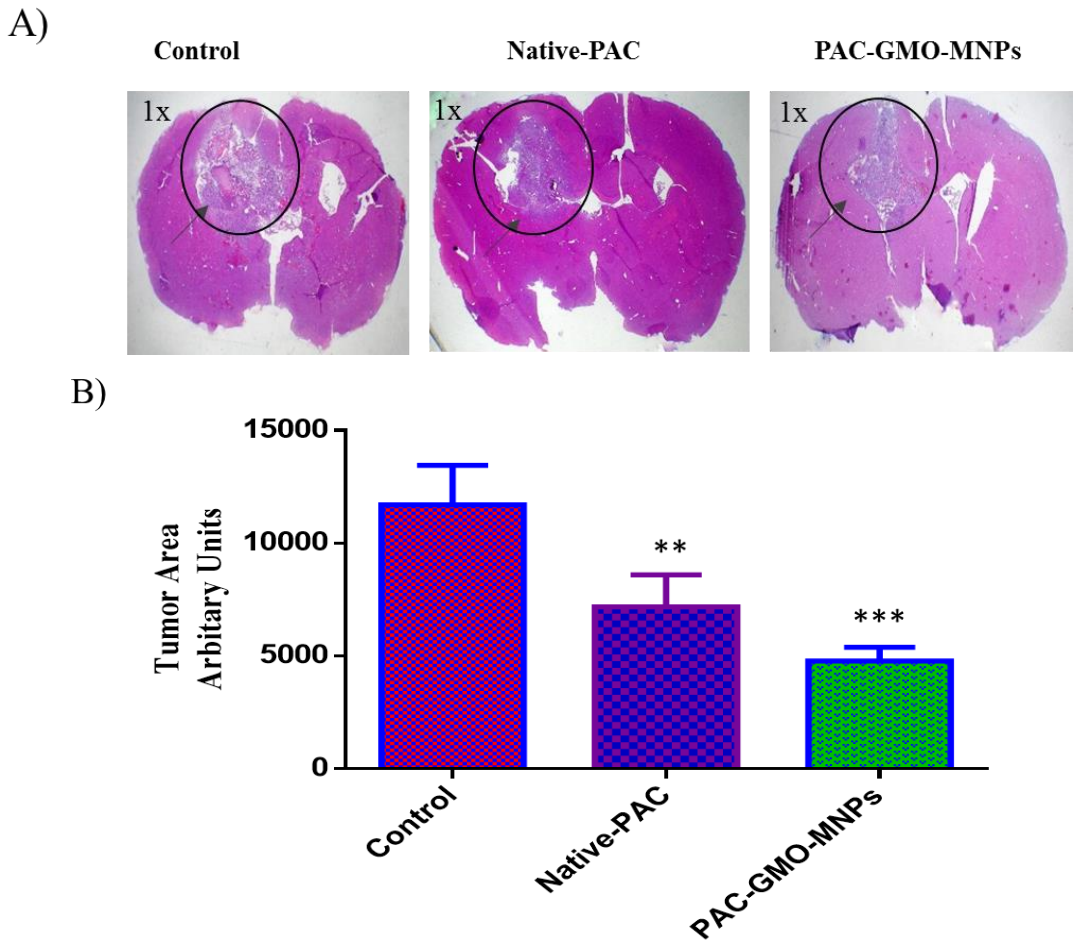

Figure 2: A) Representative pictures of H&E Histological section of glioma rats brains, where tumor regions are identified as predominate cell density with blue color which are shown in circle. Smaller tumor area were observed in PAC-GMO-MNPs group than Native-PAC and Control group. B) Control, Native-PAC and PAC-GMO-MNPs groups, each group four animals tumor areas were measured with Image J software and plotted as graph. These three groups tumor areas statistical difference were analyzed by ANOVA all pairwise comparison with Student-newman-keules, where PAC-GMO-MNPs and Native-PAC groups showing significant level smaller tumor areas compare to Control group (*** P<0.001; ** P 0.001 respectively;). The difference between Native-PAC and PAC-GMO-MNPs treated groups also found significant level difference (P 0.033).

mg/kg: 10 mg/kg respectively), after anesthesia animals were fixed on the stereotaxic frame with help of ear bars. Using a surgical blade insertion was made on head and skull was exposed. With the following stereotaxic coordinates (depth 6mm. 0.5 mm posterior, 3mm lateral from bregma), two million C6 cells in a volume of 5µl were implanted in striatum of rat. After implantation of cells skull hole was filled with dental cement and animal was removed from apparatus. 7th day of post-surgery rats were randomly divided into 4 groups, each group having four rats. Group -I received PBS, Group –II received GMO-MNPs alone (vehicle), the Group –III received native PAC and the Group –IV received PAC-GMO-MNPs. Each rat received dose of drug 3 mg/Kg body weight either in native form or equivalent concentration of drug encapsulated in MNPs formulations. Every alternative day from 7th day post-surgery onwards up to 13th day total 4 doses were administered intravenously. On 18th day, rats were sacrificed by deep anesthesia and perfused with normal saline and 4 % paraformaldehyde (PFA). Thereafter, rats were sacrificed and brains were collected in 4 % PFA. Later brains were processed in graded sucrose solution. Tumor bearing regions of the brain slice were prepared with the help of rat brain matrix and used for sectioning on saline coated slides with Leica cryostat microtome. All animal experiments were performed after institutional ethical committee (IAEC) University of Hyderabad, Hyderabad permission.

Haematoxylin and eosin staining

Sections were stained with haematoxylin and eosin by the following protocol. Sections were rehydrated with graded alcohol (100%, 95%, 70% and deionized water each step for 3 min) then stained with haematoxylin (Fisher Scientific) for 1min and washing with deionizing, tap water for 3 min. later stained with eosin (Fisher Scientific) for 45 sec, then dehydrated with graded alcohol (70 %, 95 % and 100 % for 3 min each), later sections were cleared in xylene for 3 min, mounted with DPX mount (Fine-Chem

Pvt, ltd) and observed under microscope (Olympus, CKX41, microscope digital camera, Japan).

Measurement of tumor area

Tumor bearing region **s**erial haematoxylin and eosin (H&E) stained sections were prepared from each rat brain and analyzed for the cross-sectional area that contained the largest diameter of the tumor and these tumor areas were measured with Image J software.

Immunohistochemistry

5µ thickness sections were used for immunohistochemistry analysis, prior to IHC staining tissue sections were dried for 1 hr at 60°C. Sections were rehydrated with graded alcohol and water. Antigen retrieval step was performed for 18 min (6 min x 3 times) with Tris-EDTA buffer (pH 9). Indigenous peroxidase was inhibited with peroxidase blocker as suggested by DAKO company IHC kit K0679 protocol. All Primary antibodies (Anti-KI67 #AB9260, Anti-VEGF# 05-443, Nestin # MAB353, GFAP# AB5541 were purchased from MILLIPORE and Cleaved Caspase-3 Asp175, Bcl-2, Bax antibodies were procured from Cell Signaling Technology) were used in 1:100 dilutions and incubated for 1 h at room temperature, after primary incubation sections were washed with Tris-Buffered Saline (TBS), Tris-Buffered Saline Tween (TBST) and TBS for 15 min (5 min X 3 times). Then sections were incubated with biotinylated link and streptavidin-horseradish peroxidase (HRP) as suggested in DAKO kit protocol. Further sections were counter stained with haematoxylin and mounted with Distyrene Plasticizer Xylene (DPX) mounting media and observed in Olympus light microscope attached with camera.

Statistical analysis

Statistical analysis was performed with Sigma plot 11.

RESULTS AND DISCUSSION

GBM, is one of the devastating cancers having high mortality rate because it faces unique challenge in comparison to other cancer types[19]. As GBM tumor tissues are protected externally by the skull, the treatment options primarily include surgery, radiation therapy, chemotherapy and its combinational approach. Further the treatment of GBM has been particularly ineffective due to the existence of BBB along with the blood tumor barrier (BTB) that prevents the entry and accumulation of drugs in tumor[20]. Additionally, delivery of the anticancer drugs to tumors at clinically effective concentrations by avoiding nonspecific toxicity remains a great challenge. In this regard, nanotechnology based drug delivery approach has brought a paradigm shift in the diagnosis and treatment of glioma[21]. Currently much emphasis has been given to MNPs based drug delivery system to achieve selective targeting towards the intended site and augment the therapeutic efficiency and avoid the off- target tissue toxicity[9]. MNPs as drug delivery system are unique approach for the GBM therapy. MNPs are illustrating significant potentiality as a drug delivery vehicle and represent a unique treatment option for complicated brain cancer. Paclitaxel is one of the most efficient anticancer drugs for the treatment of varieties of cancers i.e, breast, ovarian, lungs, kaposi sarcoma brain etc. Earlier our group has reported the higher cytotoxic activity of paclitaxel loaded-GMO-MNPs in the *in vitro* studies[16].

The formulated PAC-GMO-MNPs showed ~ 90% entrapment efficiency as estimated by RP-HPLC. The surface morphology of PAC-GMO-MNPs was exemplified to be having smooth and rounded topography as demonstrated by the AFM (Fig 1).

GBM animal models are developed in order to understand the disease progression, possible interference and therapeutic down regulation of tumor progression[22]. In this particular study the therapeutic efficacy of PAC-GMO-MNPs were evaluated using glioma animal model. Here the Pac-GMO-MNPs treated group illustrated significant smaller tumors as compared to Native-PAC and control (vehicle) treated group (Fig 2 A). This initial result suggested the efficacy of delivery of paclitaxel to the tumor when it is loaded into the GMO-MNPs. Apart from that, the measurement of tumor area by imageJ software depicted the reduction of tumor area in PAC-GMO-MNPs treated rats than that of native-PAC which further corroborated the effectiveness of the drug loaded MNPs formulation (Fig. 2 B).

To further validate this study we performed immunohistochemistry (IHC) staining on tissue sections with specific molecular markers related to cell proliferation, angiogenesis and cell death. The IHC analysis were done through specific molecular biomarkers, i.e antibodies that are characteristics of particular cellular events such as proliferation or cell death for the diagnosis of abnormal cells that are found in tumorigenic tissues. Cellular proliferation is the primary feature during gliomagenesis and Ki-67 is one of the classical proliferation markers for GBM. Ki-67 is a nuclear protein expressed during the various phases of the cell cycle widely and hence used as a stable marker of cell proliferation in various types of human tumors, including GBM[23]. Assessment of cell proliferation was done with a nuclear antigen Ki-67, which is basically expressed in all phases of the cell cycle except the resting G0 phase; therefore, it is a good marker for the proliferating cells[24]. Control rats have more Ki-67 nucleus stain, than that of native-PAC and PAC-GMO-MNPs. In PAC-GMO-MNP less number of nuclear stained cells were observed, illustrating the growth arrest of cells at G0 phase (Fig. 3). Glial fibrillary acidic protein (GFAP), member of cytoskeletal protein family is widely detected in GBM[25]. Therefore, GFAP was investigated as tumor marker due to its high expression in astroglial tumors, such as astrocytoma and glioblastoma[26]. There was no significant difference in the staining of GFAP in control, native-PAC and PAC-GMO-MNPs (Fig. 3).

Nestin the intermediate filament protein was another marker for invasiveness evaluated for the neural stem cells and mostly expressed in all GBMs[27]. The invasiveness of GBM is partly responsible for the shorter survival and possess a critical challenge in the treatment and management of GBM[22]. High level of nestin expression in control rats indicated invasive nature of the developed glioma, whereas, less intense staining of nestin in native-

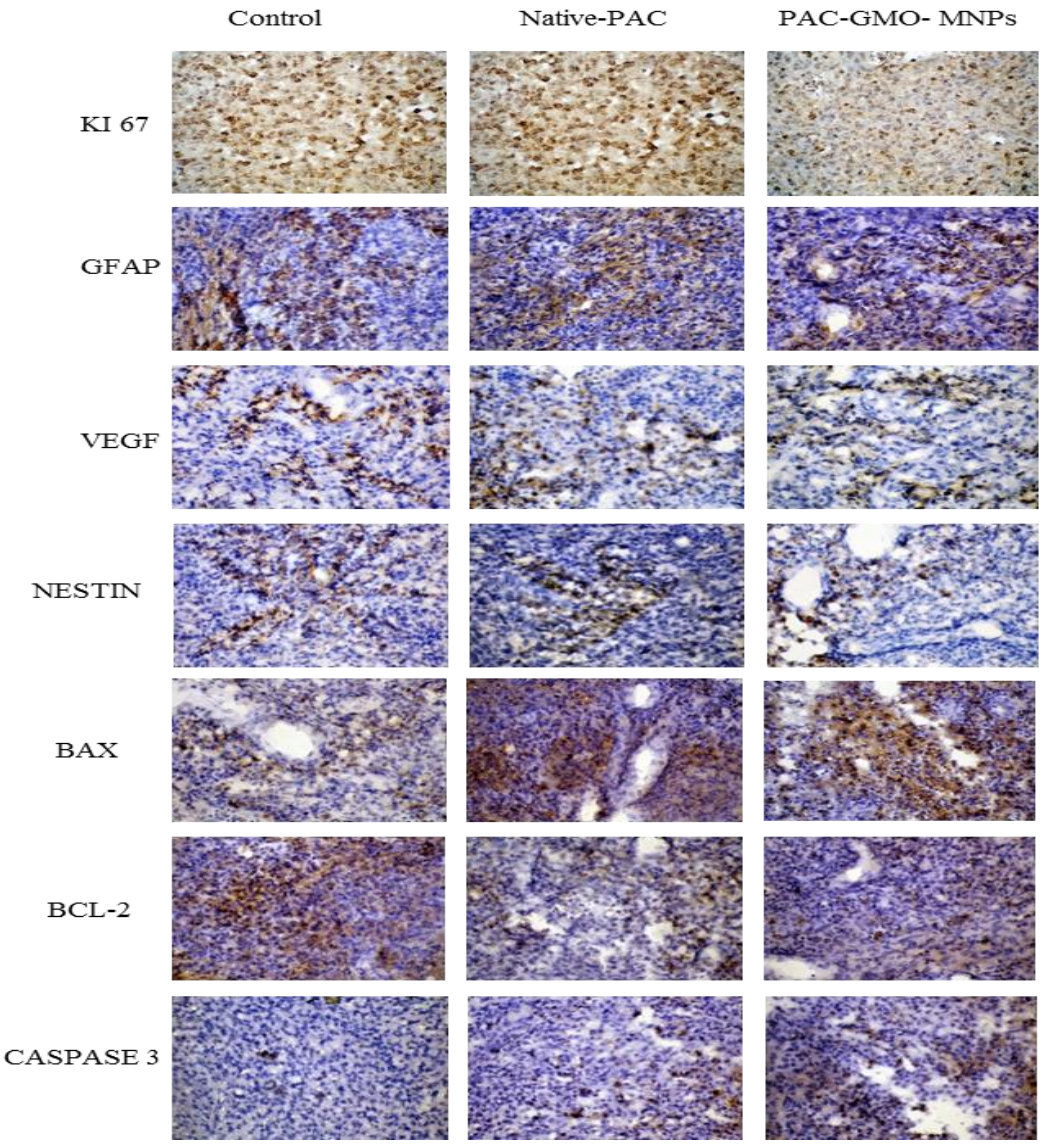

Figure 3: tumor containing sections of glioma animal treated with control, native PAC and PCA-GMO-MNPs were used for immunohitochemistry staining for tumor specific and apoptotic releted markers. Tumor specific markers, KI67 a proliferating marker, GFAP is astrocyte marker, VEGF is an agiogenic marker nestin is a glioma marker. All the tumor markers (except GFAP) show less intensity staining in native-PAC and PAC-GMO-MNPs treated animal compared to control treated animal suggesting less tumor progression observed in native-PAC and PAC-GMO-MNPs animals than control treated. Apoptosis related proteins, BAX a pro-apoptotic protein, Cleaved Caspase 3 apoptotic protein levels were increased and BCL-2 anti-apoptotic protein levels were decreased in native-PAC and PAC-GMO-MNPs treated group than control treated group.

PAC and least staining in PAC-GMO-MNPs indicated the presence of residual glioma cells probably due to tumor involution (Fig. 3). This is inconsistence with another study, wherein Bernadi et al also observed less intense nestin staining in Indomethacin loaded nanocapsules treated rats probably as a result of tumor involution[28]. Angiogenesis in tumors is caused by a number of factors, in which the vascular endothelial growth factor (VEGF) plays an important role and a potential prognostic factor for several types of cancer including GBM[29,30]. VEGF overexpression in control groups demonstrated intense staining, than that of native-PAC and PAC-GMO-MNPs. Considerable inhibition of angiogenesis in PAC-GMO-MNPs treated groups led to least staining of VEGF, depicting growth inhibition of the tumor (Fig. 3). Administration of PAC-GMO-MNPs resulted in inhibition of proliferation as well as inhibition of angiogenesis which is in agreement with nanoparticulate mediated study conducted by various groups reported the substantial inhibition of angiogenesis using doxorubicin loaded in polysorbate 80-coated poly-butylcyanoacrylate (PBCA) nanoparticles and decreased vessel density in poloxamer 188 coated doxorubicin loaded poly lactide-co-glycolide (PLGA) nanoparticles to GBM rats[30,31].

The active form of caspase-3 was explored for the detection of apoptotic event. This protease has been implicated as an effector and important marker for initiation of apoptotic event[32]. As evident from Fig. 3 the

control group of rats has less staining of caspase-3 indicating very less cell death. In native-PAC treated groups showed increased immunostaining in more number cells compared to control whereas, in PAC-GMO-MNP treated groups more number of cells were stained intensively for caspase -3 illustrating higher number of cells have undergone cell death (Fig. 3). The apoptosis is mostly regulated by the members of the Bcl-2 protein family. The inactivation of Bax leads to rapid tumor growth and the decrease extent of inactivation leads to spontaneous apoptosis of tumor cells[33]. In our observation control group and native-PAC greater numbers of cells were faintly stained, whereas in PAC-GMO-MNPs greater numbers of cells were intensely stained for Bax suggesting the activation of Bax leading to apoptosis. Whereas, in case of Bcl-2 protein, PAC-GMO-MNPs treated groups were faintly stained than that of native-PAC and control groups, implying the initiation of the apoptotic event. Taken together the intense staining of proapoptotic proteins caspase-3 and Bax protein and less intense staining of anti apoptotic protein Bcl-2 promote cell death significantly in PAC-GMO-MNP treated glioma induced rat model (Fig. 3). Our findings are in line with previous reports of targeted efficacy of surfactant coated drug loaded nanoparticles in the brain against various disease including the GBM[4,30,31,34]. Nance et al, observed delayed tumor growth of GBM using nanoformulation of paclitaxel loaded in poly(lactic-co-glycolic acid) (PLGA)-co-PEG block copolymer system by local targeting to the brain[35]. Utilization of PAC-GMO-MNPs formulation as chemotherapy for GBM successfully demonstrated that this drug loaded MNPs have efficiently crossed the BBB and endocytosed in an effective concentration, which led to enhanced cell death activity. Earlier, Dilnawaz et al, have demonstrated the developed PAC-GMO-MNPs has crossed the BBB in the normal healthy rats[18]. However, in our study unique formulation of the non-surfactant amphiphilic glyceryl monooleate coating impersonated the role of low-density lipoprotein in the brain endothelial cells and in turn accumulated enormously in the GBM rats and triggered cell death. Therefore, the developed MNPs have the potential ability for the GBM therapy in future clinical studies.

CONCLUSIONS

Administered PAC-GMO-MNPs with high drug loading capacity demonstrated enhanced therapeutic efficacy as observed in a GBM rat model using H&E and IHC analysis approach as compared to native paclitaxel. Therefore, we envisage that this drug loaded GMO-MNPs could play an essential role in the treatment of chemotherapy.

ACKNOWLEDGEMENTS

Financial support of Department of Science and technology (DST)-Nano UoH project, DST-SERB, Department of Biotechnology (DBT) India, and Council of Scientific and Industrial research (CSIR) India is acknowledged. RC is grateful for the financial support of Union Grant Commission (UGC) India for student fellowship. FD greatly acknowledges Department of Science and Technology, Government of India for the financial support in form of women scientist grant (SR/WOS-A/LS-136/2010).

REFERENCES

1. CH Fan, CY Ting, HJ Lin, CH Wang, HL Liu, TC Yen, CK Yeh: SPIO-conjugated, doxorubicin-loaded microbubbles for concurrent MRI and focused-ultrasound enhanced brain-tumor drug delivery. *Biomaterials* 2013, 34:3706-15.
2. F Ye, A Barrefelt, H Asem, M Abedi-Valugerdi, I El-Serafi, M Saghafian, K Abu-Salah, S Alrokayan, M Muhammed, M Hassan: Biodegradable polymeric vesicles containing magnetic nanoparticles, quantum dots and anticancer drugs for drug delivery and imaging. *Biomaterials* 2014, 35:3885-94.
3. D Brat, Castellano-Sanchez A, Kaur B, Van Meir EG.: Genetic and biologic progression in astrocytomas and their relation to angiogenic dysregulation. *Adv Anat Pathol* 2002, 9:24–36.
4. J Kreuter: Nanoparticulate systems for brain delivery of drugs. *Adv Drug Deliv Rev.* 2001, 47:65-81.
5. V Roullin, Mege M, Lemaire L, et al.: Influence of 5-fluorouracil-loaded microsphere formulation on efficient rat glioma radiosensitization. . *Pharm Res* 2004, 21:1558–1563. .
6. GD Chen, H.A.E. Benson, : Drug delivery across the blood– brain barrier,. *Cur Drug Deliv.* 2004, 1:361–376.
7. SB Tiwari, Amiji, M.S.: A review of nanocarrier-based CNS delivery systems. *Curr. Drug Deliv.* 2006, 3: 219-232.
8. S Wohlfart, Gelperina, S, Kreuter, J. : Transport of drugs across the blood-brain barrier by nanoparticles. *J Control Release.* 2012,, 161:264-73.
9. A Singh, Sahoo, SK.: Magnetic nanoparticles: a novel platform for cancer theranostics. *Drug Discov Today.* 2014, 19:474-81.
10. B Chertok, Moffat, BA, David AE, Yu F, Bergemann C, Ross BD, Yang VC.: Iron oxide nanoparticles as a drug delivery vehicle for MRI monitored magnetic targeting of brain tumors. *Biomaterials* 2008, 29:487-96.
11. S Pulfer, Ciccotto, SL, Gallo, JM. : Distribution of small magnetic particles in brain tumor-bearing rats. *J. Neurooncol.* 1999, 41:99–105.
12. K Widder, Marino PA, Morris RM, Howard DP, Poore GA, Senyei AE. : Selective targeting of magnetic albumin microspheres to the Yoshida sarcoma: ultrastructural evaluation of microsphere disposition. *Eur J Cancer Clin Oncol.* 1983, 19:141-7.
13. J Koziara, Lockman, PR, Allen DD, Mumper RJ. : Paclitaxel nanoparticles for the potential treatment of brain tumors. *J Control Release* 2004, 99:259–69.
14. Y Zhao, E Butler, M Tan: Targeting cellular metabolism to improve cancer therapeutics *Cell Death and Disease* 2013, 4:e532.
15. X Ke, Zhao, BJ, Zhao X, Wang Y, Huang Y, Chen XM, Zhao BX, Zhao SS, Zhang X, Zhang Q. : The therapeutic efficacy of conjugated linoleic acid -

paclitaxel on glioma in the rat. *Biomaterials.* 2010, 31:5855-64.

16. F Dilnawaz, Singh, A, Mohanty, C, Sahoo, SK.: Dual drug loaded superparamagnetic iron oxide nanoparticles for targeted cancer therapy. . *Biomaterials.* 2010, 31:3694-706.
17. FaS Dilnawaz, SK. : Enhanced accumulation of curcumin and temozolomide loaded magnetic nanoparticles executes profound cytotoxic effect in glioblastoma spheroid model. *Euro. J. Pharmaceu and Biopharmaceu.* 2013, 85:452–462.
18. F Dilnawaz, Singh, A, Mewar S, Sharma U, Jagannathan NR, Sahoo SK.: The transport of non-surfactant based paclitaxel loaded magnetic nanoparticles across the blood brain barrier in a rat model. . *Biomaterials.* 2012, 33:2936-51.
19. H Gao, Z Yang, S Cao, Y Xiong, S Zhang, Z Pang, X Jiang: Tumor cells and neovasculature dual targeting delivery for glioblastoma treatment,. *Biomaterials* 2014, 35:2374–2382.
20. C Zhan, Lu, W: The blood-brain/tumor barriers: challenges and chances for malignant gliomas targeted drug delivery. *Curr Pharm Biotechnol.* 2012, 13:2380-7.
21. J Wojton, Z Chu, H Mathsyaraja, W Meisen, N Denton, C-H Kwon, L Chow, M Palascak, R Franco, T Bourdeau, et al: Systemic Delivery of SapC-DOPS Has Antiangiogenic and Antitumor Effects Against Glioblastoma. *Molecular Therapy* 2013, 21: 1517–1525.
22. T Strojnik, Røsland, GV, Sakariassen, PO, Kavalar, R, Lah, T. : Neural stem cell markers, nestin and musashi proteins, in the progression of human glioma: correlation of nestin with prognosis of patient survival. *Surg Neurol.* 2007, 68:133-43.
23. JW Qiang, Zhang, Xiao-guang, Q, Wei, Y, Gan,Y, Yan-wei,L,Tao, J and Lei,W: Gene expression profiling reveals Ki-67 associated proliferation signature in human glioblastoma. *Chin Med J* 2011, 124:2584-2588.
24. J Gerdes, Lemke, H, Baisch, H, Wacker, HH, Schwab, U, Stein, H. : Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol.* 1984, 133:1710-5.
25. C Jung, Foerch C, Schänzer A, Heck A, Plate KH, Seifert V, Steinmetz H, Raabe A, Sitzer M.: Serum GFAP is a diagnostic marker for glioblastoma multiforme. *Brain.* 2007, 130:3336-41.
26. K Hamaya, Doi, K, Tanaka, T, Nishimoto, A: The determination of glial fibrillary acidic protein for the diagnosis and histogenetic study of central nervous system tumors: a study of 152 cases. *Acta Med Okayama* 1985, 39:453–462.
27. C Wiese, Rolletschek, A, Kania, G, Blyszczuk P, Tarasov KV, Tarasova Y, Wersto RP, et al.: Nestin expression: a property of multi-lineage progenitor cells? *Cell Mol Life Sci* 2004, 61:2510–2522. .
28. BE Bernardi A, Jäger E, et al.: Indomethacin-loaded nano¬capsules treatment reduces in vivo glioblastoma growth in a rat glioma model. . *Cancer Lett* 2009, 281:53–63.
29. S Brem, Contran, R, Folkman, J. : Tumor angiogenesis: A quantitative method for histologic grading. . *J Nat Cancer Inst* 1972, 48:347-356.
30. S Wohlfart, A Khalansky, S Gelperina, O Maksimenko, C Bernreuther, M Markus Glatzel, J Kreuter: Efficient chemotherapy of rat glioblastoma using doxorubicin-loaded plga nanoparticles with different stabilizers. . *PLoS ONE* 2011, 6: e19121. .
31. T Hekmatara, Bernreuther, C, Khalansky, AS, Theisen, A, Weissenberger J, et al.: Efficient systemic therapy of rat glioblastoma by nanoparticle-bound doxorubicin is due to antiangiogenic effects. . *Clin Neuropathol* 2009, 28:153–164.
32. J Walsh, S Cullen, C Sheridan, A Lüthi, C Gerner, S Martin: Executioner caspase-3 and caspase-7 are functionally distinct proteases,. *PNAS* 2008, 105:12815–12819.
33. C Yin, Knudson, CM, Korsmeyer, SJ, Dyke, TV. : Bax suppresses tumorigenesis and stimulates apoptosis in vivo. . *Nature Rev Genet.* 1997, 385:637–640.
34. BB Petri, A.; Khalansky, A.; Hekmatara, T.; Muller, R.;Uhl, R.; Kreuter, J.; Gelperina, S.: Chemotherapy of Brain Tumour Using Doxorubicin Bound to Surfactant-Coated Poly(butyl cyanoacrylate) Nanoparticles: Revisitingthe Role of Surfactants. *J. Controlled Release* 2007, 117:51–58.
35. ZC Nance E, Shih TY, Xu Q, Schuster BS, Hanes J.: Brain-penetrating nanoparticles improve Paclitaxel efficacy in malignant glioma following local administration.. *ACS Nano* 2014, 8:10655-64.