11.1 Introduction

The brain tumors in human are very challenging due to the difficulties of identifying early cancer lesions and little effective treatments. Out of all central nervous system tumors, gliomas account for about half. Gliomas are difficult to be radically cured because of its invasive growth and other malignant behaviors. Apart from this, most of gliomas are difficult to be early detected, even they are discovered, is difficult to cure them because of their resistance to radiation or chemotherapy. Therefore, developing animal models of human gliomas is essential to explore the mechanisms of occurrence and development of brain tumor and promote clinical research (1). To verify the activity of the any compound or formulation *in vitro* and *in vivo* studies both are essential. The *in vitro* cell line screening techniques provide faster results in a cost effective manner but at the same time inadequate to estimate the off target effects which may contribute to the potency or toxicity of the novel formulation (2). Animal studies are critical for understanding the fundamental processes that support in vivo tumor development as tumor cells grown in vitro are not necessarily analogous to those that develop in a human subject (3).

There are several types of brain tumor models which are summarized below (1):

- Allogeneic graft model of mouse brain tumor
- Xenograft model of human brain tumor
- Genetic engineering model of mouse brain tumor
- Genetic engineering mouse of medulloblastoma

Out of all these Xenograft model of human brain tumor was utilized for the studies.

11.2 Cell line

U-87 MG cell line was obtained from the National Centre for Cell Sciences (NCCS, Pune, India) and used for induction of tumor in vivo. Cells were grown in DMEM supplemented with L-glutamine (2 mM), 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution (100 µg/ml streptomycin, 100 units/ml penicillin and 50 µg/ml amphotericin). The culture was maintained at 37 ºC under a humidified atmosphere with 5% CO2. The culture medium was changed at regular time intervals.

11.3 Ethical statement

The in vivo tumor regression analysis was carried out by Deshpande Laboratories Pvt. Ltd. (Bhopal, India) under the protocol approval number DL/PR/05/20. All the experimental procedures were carried out as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines released by Ministry of Environment, Forests and Climate Change and Ministry of Social Justice and Empowerment, Government of India, New Delhi, India.

11.4 Animals

In bred balb/c mice (8-10 weeks old) were used in the study. Mice were housed in individually ventilated cage system and fed with standard sterilized diet and sterilized water ad libitum. Animals were maintained in 12h-12h light- dark cycle, 25 °C air conditioned and controlled noise rooms.

11.5 Method

Balb/c mice (8-10 weeks) were used for the experiment (4,5). Briefly, U87 MG cells (cell density = 1×10^5) were injected on the back of the mice and allowed to form tumors. The animals were divided into three groups (n = 3): Group I (model control; untreated group), Group II (Standard control; LND) and Group III (Lf-LNPs). After reaching the tumor a palpable size (100 ± 10 mm), 0.9 % saline solution, pure LND (3 mg/kg) and Lf-LNPs equivalent to 3 mg/kg of LND were administered to group I, group II and group III respectively via i.v. route once a week. Tumor volume was measured using digital vernier calipers (Mitutoyo JAPAN). Tumor volume was calculated by the below mentioned equation:

$$Volume = \frac{Width^2 \times Length}{2} \quad \text{.....Equation 11.1}$$

At the end of the experiment the animals were sacrificed by overdose of thiopentone sodium. The animals were dissected and tumors were excised. The excised tumors were immediately imaged.

11.6 Statistical analysis

All the results are expressed as mean ± SEM and mean ± SEM. The data were analyzed using GraphPad Prism 7 software. Multi-group comparisons of the means were carried out by one way analysis of variance (ANOVA) test. $P<0.05$ were considered statistically significant.

11.7 Results and discussion

In vivo tumor regression study was performed to assess the anticancer activity of developed Lf-LNPs with respect to pure LND (standard control). The results of tumor volume and percent tumor growth are summarized in table 11.1 and 11.2 respectively.

Table 11.1: Changes in the tumor volume of untreated (model control), LND (standard control) and Lf-LNPs treated mice

	Model control	**Standard control (LND)**	**Lf-LNPs**
Days	Mean ± SEM	Mean ± SEM	Mean ± SEM
0	53.77 ± 7.77	44.70 ± 3.01	64.50 ± 10.09
5	70.17 ± 7.09	46.70 ± 3.00	66.57 ± 9.99
10	89.13 ± 6.38	63.87 ± 7.18	76.60 ± 7.66
15	114.70 ± 4.35	73.43 ± 2.97	79.07 ± 7.65
20	152.03 ± 28.08	86.00 ± 11.15	99.40 ± 3.78
25	179.93 ± 25.91	114.17 ± 14.78	104.50 ± 7.14
30	213.17 ± 25.77	112.97 ± 5.21	136.80 ± 6.98

The percent tumor growth results indicated significant tumor growth in all the groups at the end of the study. As compared to model control, standard control (LND treated) and Lf-LNPs treated group exhibited less % tumor growth. At the end of the study, 4.41 fold, 2.56 folds and 2.22 folds increase in percent tumor growth was observed for model control, standard control and Lf-LNPs treated group respectively. The results of Lf-LNPs indicated least increase in the percent tumor growth as compared to model control and standard control which indicated better

anticancer potential of Lf-LNPs as compared to LND (figure 11.2). The obtained results may be due to encapsulation of drug inside the surface modified NPs. Lf coating provide the target specific drug delivery of LND towards U-87 MG cells induced tumor. Apart from BBB, the Lf receptors are also over expressed in glioma cells and surface modification of LNPs with Lf enhanced the LND concentration at the tumor site by targeting Lf receptors. This led to more inhibition of tumor growth as compared to pure LND. The results of ANOVA analysis also indicated significant difference in the obtained results with the p value = 0.0102 (p value < 0.05).

Table 11.2: Percent tumor growth of untreated (model control), pure LND (standard control) and Lf-LNPs treated mice.

	Model control	**Standard control (LND)**	**Lf-LNPs**
Days	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
0	100.00 ± 0	100.00 ± 0	100.00 ± 0
5	138.27 ± 31.29	104.57 ± 1.33	103.50 ± 2.87
10	174.80 ± 34.81	145.50 ± 23.55	121.67 ± 10.14
15	221.37 ± 29.44	166.67 ± 17.48	125.90 ± 11.51
20	282.70 ± 27.19	197.70 ± 40.17	161.23 ± 22.78
25	348.13 ± 71.53	260.90 ± 46.40	175.57 ± 43.84
30	413.83 ± 82.68	255.97 ± 26.05	222.40 ± 33.17

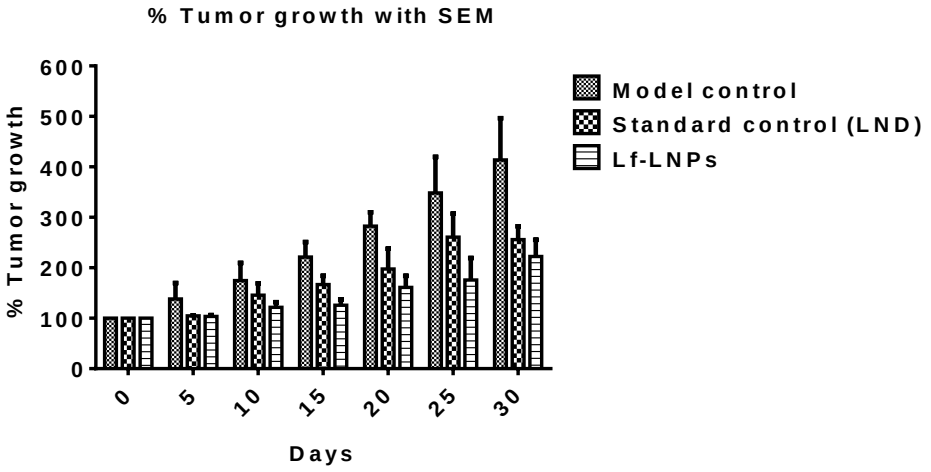

Figure 11.2: Graphical representation of average changes in the % tumor growth of untreated (model control), pure LND (standard control) and Lf-LNPs treated mice. Error bars represent SEM, (n=3)

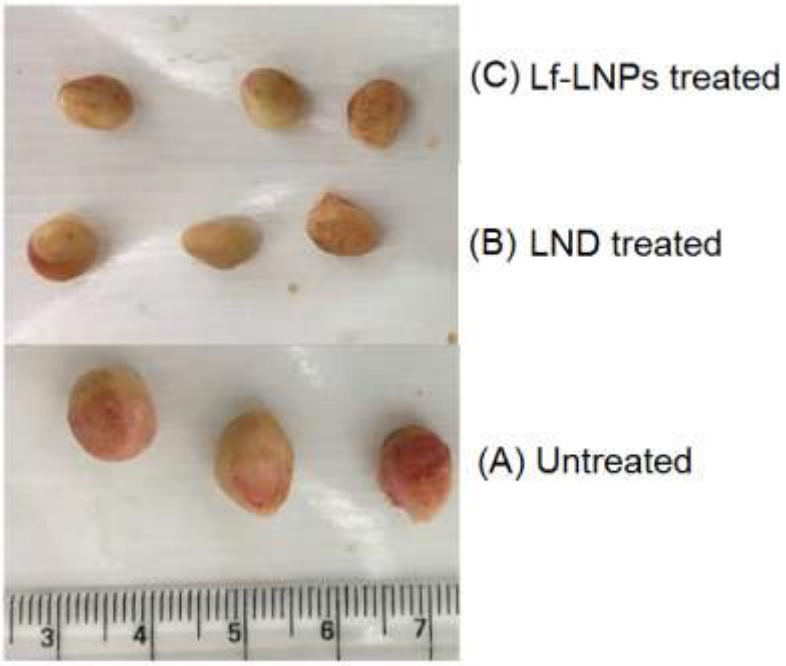

Figure 11.3: Excised tumor image of the animals treated with different test sample (A) untreated group (saline treated), (B) standard control (LND treated) and (C) Lf-LNPs treated after 30 terminal of study (three each)

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