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Ofloxacin targeting to lungs by way of microspheres

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ABSTRACT

The efficacy of drug candidates is frequently limited by their inability to reach the target site of action, especially when they are administered through conventional dosage forms or drug delivery systems. Targeted drug delivery systems have increased the amount of drug reaching the site and simultaneously decrease the amount being distributed to other parts of the body. Microspheres have emerged as a remedial measure to improve site-specific drug delivery to a considerable extent. As an application, lung-targeting albumin loaded ofloxacin microspheres (ALOME) were prepared by water in oil emulsion method. The appearance and size distribution were examined by scanning electron microscopy, and the aspects such as in vitro release characteristics, stability, drug loading, loading efficiency, pharmacokinetics and tissue distribution in albino mice were studied. The experimental results showed that the microspheres have an average particle size of 11.32 μm. The drug loading and loading efficiency were (66.95 and 94.8%) respectively. The in vitro release profile of the microspheres matched the Korsmeyer's Peppas release pattern, and the release after 1 h was 42%, while for the original drug, ofloxacin, under the same conditions, 90.02% released in the first half an hour. After intravenous administration (15 min), the drug concentration of microspheres group in lung of albino mice was 432 μg g^{-1} while that of controlled group was 1.32 μg g^{-1} ALOME found to release the drug to a maximum extent in the target tissue, lung. Histopathological studies proved the tissue compatibility of ALOME to be safe.

1. Introduction

For a large majority of pharmaceutical formulations in contemporary use, their 'specificity' towards appropriate sites of a disease is not based on their ability to accumulate selectively in the target organ or tissue. Usually, they are more or less evenly distributed within the body. Moreover, to reach the target area, the drug has to cross-different biological barriers—organs, cells, even intracellular compartments, where it can cause undesirable side-reactions, or be partially inactivated. The best solution to this issue is drug targeting (Shixuan et al., 2009; Jorg, 1994).

Ofloxacin of late has emerged as the gold standard in the treatment of pneumonia, nevertheless the conventional approaches adopted to administer ofloxacin by oral or parenteral routes does not provide an efficacious mode of treatment (Yuk et al., 1991).

Exploring alternative routes of administration like microspheres to develop a targeted drug delivery system and to act locally on the organ of infection will improve the therapeutic efficacy, reduce side effects and thereby provide patient compliance (Illum et al., 1982; Delgado et al., 2000; Dhanaraj et al., 2001).

In this work, the targeting efficiency of ofloxacin microspheres to lungs was 91.7% when injected into the vein of albino mice, and was found to have a sustained release profile.

2. Materials and methods

2.1. Experimental

2.1.1. Preparation of ofloxacin albumin microspheres

The albumin microspheres were prepared by taking 2 g of the ofloxacin and 2 g of albumin (1:1 ratio). This was solubilized (25 mL of water), then the contents were slowly added to a beaker containing 65 mL of sesame oil NF (Voigt Global) and stirred for 1 h at 1000 rpm. To this, 0.5 mL of Tween 80 (Sigma–Aldrich) was added and was stirred at temperature of 40 °C to ensure the hardening process for 25 min. The resulting microspheres were stabilized by cross-linking using 0.1 mL 25% m/V glutaraldehyde (Sigma–Aldrich) solution for a period of 15 min. The microspheres were collected by filtration and washed with n-hexane and dried at room temperature (Pandey et al., 2002).

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2.2. Materials

Albumin purchased from Sigma Chemicals, India, was used as carrier. Ofloxacin (content 99.85%) was provided by Micro Labs, Hosur, India. Other reagents used were of analytical grade.

2.3. Surface morphology

The surface morphology of the microspheres was observed by scanning electron microscope (Joel, Japan). The microspheres were placed on graphite surface and coated with gold using an ion sputter (Joel) and were observed at 12 kV (Canan et al., 2003).

2.4. Particle size distribution

The particle size distribution of the microspheres was studied by laser light diffraction and ultrasonic techniques (HELOS laser system for dry powders, Sympatec, Inc., Germany) (Hai et al., 2002).

2.5. Determination of ofloxacin drug loading

A weighed quantity of microspheres was dissolved in 0.1 M HCl. The ofloxacin content was assayed by spectrometry at 297 nm by constructing a calibration curve (Jia et al., 1997) (UV-1601 Spectrophotometer, shimadzu). The best fit straight line equation of the calibration curve was found to be $A = 0.314x + 0.0063$. The microsphere yield was 94.8%. The experiments were conducted in triplicate. The average value of ofloxacin content was designated the drug loading. The drug loading was calculated (Lu et al., 2003) as a ratio between the amount of ofloxacin loaded and the theoretical amount of ofloxacin loaded for each formulation:

$$\text{drug loading}\,(\%) = \frac{Q_m}{W_m} \times 100$$

where Q_m is the amount of ofloxacin in each composition and W_m is the theoretical amount of ofloxacin in each composition calculated from the quantity added in the microsphere prepartion process.

2.6. In vitro dissolution study of ALOME

In vitro release patterns were studied using conventional dialysis technique. Ofloxacin albumin microspheres were placed in a dialysis bag and dialyzed against 200 mL of phosphate saline buffer (PBS). The PBS was prepared by adding 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 to 800 mL water the pH was adjusted to 7.4 with HCl and the volume was made up to 1000 mL. During the dissolution process the temperature was stabilized at 37 ± 1 °C, and the sink conditions were maintained throughout the course of study (Sree and Shobha, 2006). The in vitro release was aided by continuous stirring using a magnetic stirrer.

2.7. Stability of ALOME

The stability protocol was designed based on the ICH Q2B Guidelines. The microspheres were stored at 25 ± 2 °C and 60 ± 5% relative humidity (RH) for a period of 6 months and at 5 ± 3 °C for a period of 12 months, which is the accelerated storage temperature and long-term storage temperature respectively for products which are intended to be refrigerated. The decision to refrigerate was taken because of the thermosensitivity and thermolability of the polymer (Lacoulonche et al., 1999; Svein et al., 1997; Sree and Shobha, 2006). Since albumin is of protein origin and has relatively low thermal stability profile, it is prone to degeneration at 25 °C. The stored samples were tested for their drug content, particle size distribution, and for any physical change. The drug content was determined by a validated UV spectrophotometric method.

The testing was carried out at 0, 2, 4 and 6 months (as per FDA's Guideline for submitting documentation or stability of human drugs and biologics DHHA, February 1987) for accelerated storage condition and at 3-month interval for a period of 12 months for long-term storage condition as per ICH guideline (ICH Q6A Test procedures and acceptance criteria for new drug substance and new drug products).

2.8. In vitro pharmacokinetics of ALOME

Eight-week old, healthy, laboratory-bred, Swiss Albino mice (*Mus musculus*), of either sex, weighing 20 ± 2 g were maintained under conventional laboratory conditions at temperature 25 ± 2 °C and a 12 h natural light period. Commercial pellet diet (Gold Mohur, Lipton India) and tap water were provided ad libitum. The experiments were conducted at CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals, Chennai, India) approved animal house.

Thirty-six adult albino mice were used. Prior to administration, the animals were randomly divided into 6 groups with 6 animals in each group to test the drug release at the following time periods: 10 min, 30 min, 1 h, 3 h, 6 h, and 12 h. One group was administered 520 μg kg^{-1} ofloxacin injection via the tail vein, while the other group received equivalent amount of drug in ALOME suspension form. All the animals were kept on starvation 12 h before injection, with free access to water. At predetermined time intervals as stated earlier, the animals were injected with the microspheres via the tail vein and were sacrificed by cervical dislocation. The organs that were studied for target action, viz. lungs, liver and spleen were extracted (Zhang et al., 1992). The tissue samples were stored for 24 h at −20 °C. Then the concentration of drug localized in each organ as the result of targeted release was determined through extraction method.

The extracted organs were homogenized by adding saline in the concentration of 0.1 mg mL^{-1}. The protein in the mixture was precipitated (to prevent interference during the quantification process) by adding 2 mol L^{-1} perchloric acid (100 μL). The mixture was then vortexed for 5 s to aid the mixing process and was then centrifuged at 5000 rpm for 15 min (Tadashi and Kazunobu, 1992; Masakazu et al., 1994; Himangshu et al., 2004; Sree and Shobha, 2006). The prepared samples were used to determine the amount of ofloxacin present in the tissue samples quantitatively using high-performance liquid chromatography (HPLC) method (Huo et al., 2005).

2.9. Tissue distribution of microspheres

All animals were kept on starvation 12 h before injection, with free access to water. A dose of 520 μg kg^{-1} was administered to each animal as dispersion in saline with 1% of Tween 80. The animals were injected with ALOME via tail vein and were sacrificed by cervical dislocation. The organs that were studied for target action, viz. lungs, liver and spleen were extracted. The tissue samples were stored for 24 h at −20 °C and then 1 g of tissue sample was collected respectively after surface water was dried. The drug concentration in tissues was determined by HPLC quantification method (Huo et al., 2005).

2.10. Evaluation of lung-targeting of ALOME

Three targeting parameters (r_e, time-average relative drug exposure; drug targeting efficiency, t_e; overall drug targeting efficiency of a delivery system against non-target tissue, T_e) were calculated for evaluation of lung-targeting characteristics (Gupta and Hung, 1989).

2.11. Histopathological studies

After 12 h of administration of the formulation, animals (including control group) were sacrificed by excess anesthesia and the lungs, liver and spleen were dissected and washed with cold saline. The organs were pressed between filter pads and weighed. Lung, liver and spleen tissues were fixed in 10% neutral formalin using standard techniques and stained with hematoxylin and eosin for histopathological examination. All tissue samples were examined and graded under light microscopy with 500× magnification (Nanji et al., 2002).

2.12. Statistical analysis

The data was statistically analysed by Student's *t* test. A *P* value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Data obtained from experimental results

Microspheres prepared were smooth, discrete with a regular spherical to near-spherical shape. Examination using scanning electron photomicrographs showed spherical particles with smooth surfaces, which may be due to cross-linking of coacervate to stabilize emulsion droplets and hence resulted in well-formed microspheres Fig. 1. The drug loading and the percentage yield were 67 ± 0.3 and 94.8 ± 0.2 respectively.

3.2. Appearance, particle size and distribution

The particle size distribution is an important factor since it controls the tissue location of the microspheres after their intra-arterial infusion. Previous reports pointed out that the microspheres with the size range of 5–25 μm have a notable lung-targeting (Kanke et al., 1980). The average particle size was 11.32 μm. The results showed that the microspheres were mainly accumulated in the lung after intravenous injection to the albino mice (Fig. 2a and b).

3.3. In vitro release and release pattern

Fig. 3 shows in vitro release for ALOME and ofloxacin, respectively. About 42% of the total ofloxacin in ALOME was released in the first hour, which reflected the significant amount of ofloxacin adsorbed on or incorporated near the surface of the microspheres.

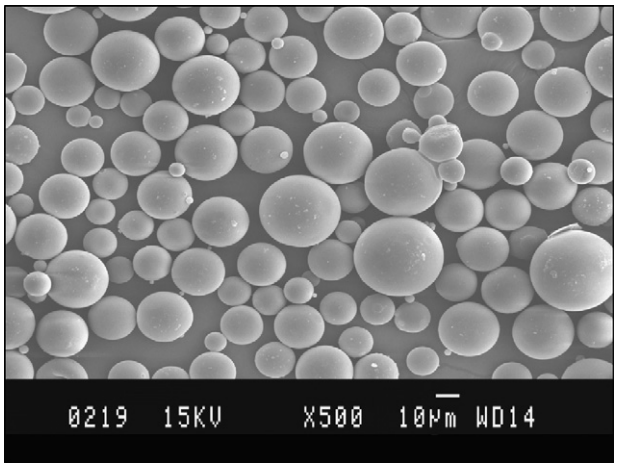

Fig. 1. Scanning electron microscopy of ofloxacin albumin microspheres prepared by water in oil emulsion method. The photograph was taken at 500× mangification.

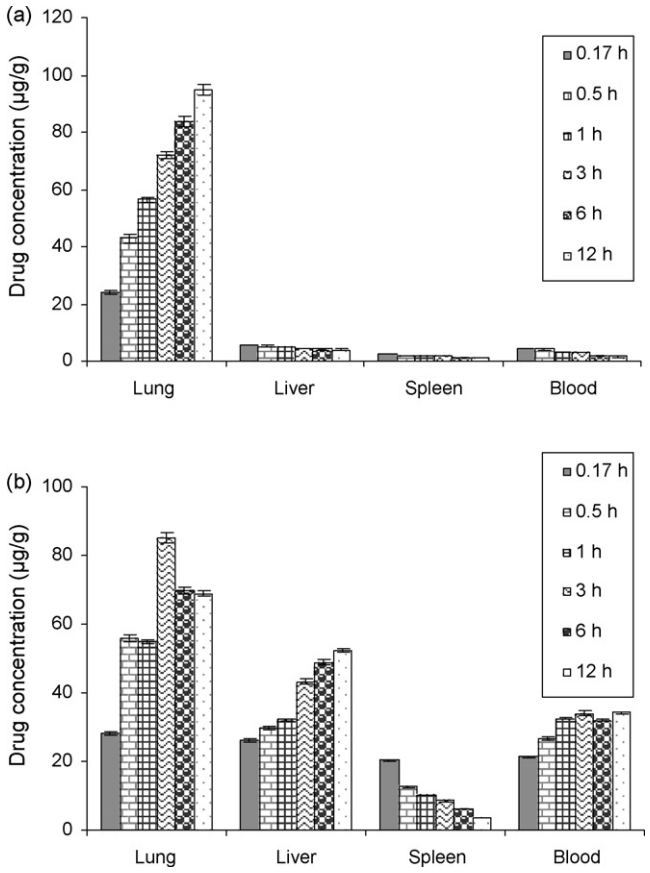

Fig. 2. (a) Distribution in tissue in albino mice after injection of ALOME in tissue (μg g^{-1}) and blood (μg/mL). (b) Distribution in tissue in albino mice after injection of ofloxacin in tissue (μm/g) and blood (μg/mL) (bars represent mean ± SD; $n = 3$).

In clinical practice this would lead to 'burst effect', which enables the preparation to show fast effect to the patients. However, the ofloxacin release from albumin microspheres was completed in 6 h. During this period, the released amount was 99.3%. In comparison with ALOME, the ofloxacin injection releases ofloxacin very fast; 90% of ofloxacin has been released in 0.5 h. The results indicated that ALOME had a well-controlled release efficacy.

The data obtained from in vitro release studies fitted to various kinetic equations (i.e., first-order, Baker and Lonsdale, Hixon and Crowell, Korsmeyer's Peppas and Higuchi (Sree and Shobha, 2006) to determine the mechanism of drug release and release rate using a macrowritten for graphing tool sigma plot version 9.01 Fig. 4. The correlation coefficient value, R^2, is taken into account to decide

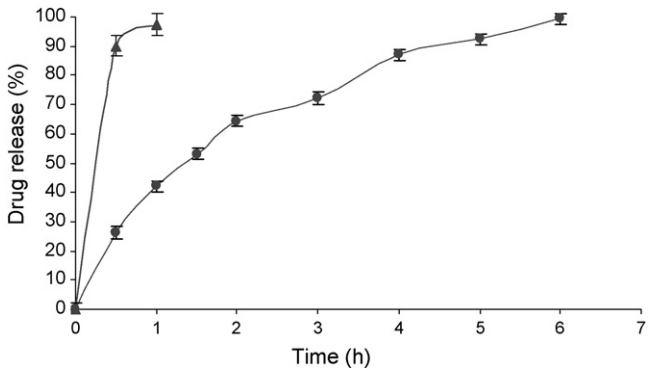

Fig. 3. Cumulative amount of drug released (▲) ofloxacin injection (■) ALOME (bars represent mean ± SD; $n = 3$).

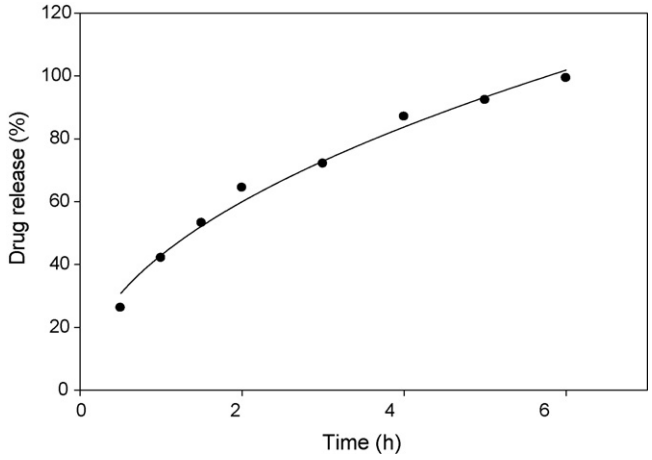

Fig. 4. In vitro release profile of ALOME—curve fit for Korsmeyer's Peppas model (bars represent mean ± SD; $n = 3$).

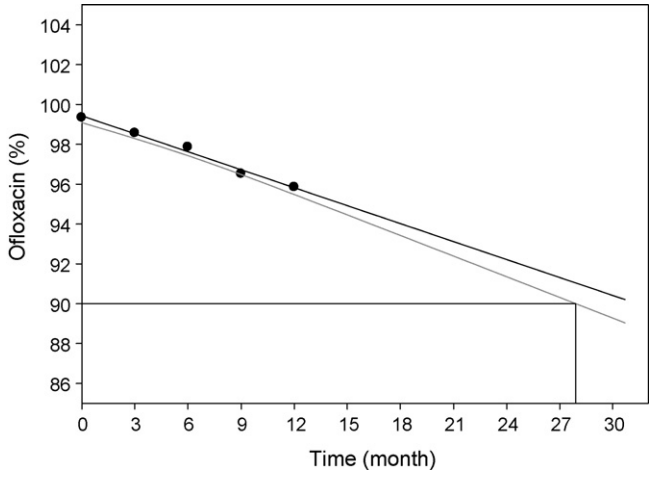

Fig. 5. Drug content of ALOME under accelerated storage conditions.

upon the relevance of the model/curve fit which will best describe the extent of fit. According to the R^2 values given by different data fits for ALOME, the Korsmeyer's Peppas model was to be an ideal fit having $R^2 = 0.9968$. According to Korsmeyer's Peppas fit, the release of the drug is decided upon by the diffusion of the polymeric matrix and the drug release is governed by Fick's law of diffusion.

The release pattern showed classical Fickian diffusion; aided by the initial swelling of microspheres and releasing drug particles adsorbed on the surface aiding in a 'burst' release (Lu et al., 2003). Later on, the release pattern is more controlled and sustained for longer period of time due to diffusion.

3.4. Test for stability

The drug content in long-term storage conditions did not vary to a large extent in the microsphere formulations; the maximum

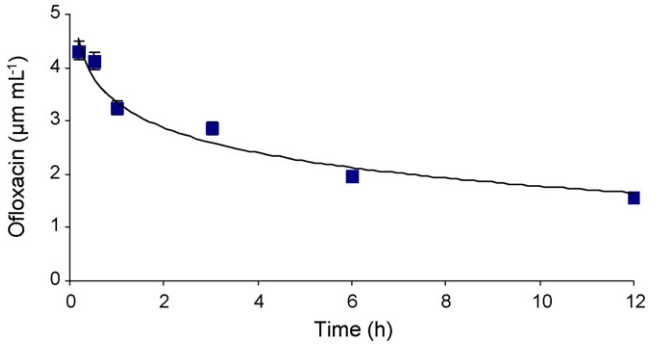

Fig. 6. Two-compartment model following i.v. administration for ALOME (■) observed, (—) predicted ($n = 3$; bars denote SD values).

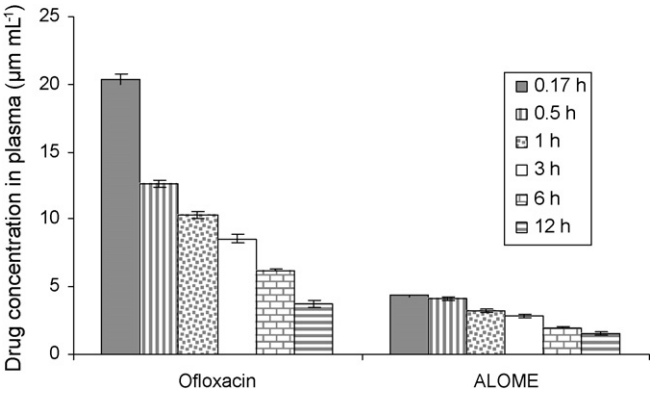

Fig. 7. Plasma drug concentration of ofloxacin injection and ALOME at different time intervals (bars represent mean ± SD; $n = 3$).

Table 2
Pharmacokinetics parameters of ALOME group and ofloxacin group in thirty number of albino mice. Data are presented as means ± SD ($n = 3$).

Parameters	ALOME	Ofloxacin injection
AUC (μg h/mL^{-1})	37.34	133.70
$t_{1/2}$ (α) (h)	0.95	0.37
$t_{1/2}$ (β) (h)	21.9	10.7
K_{21} (h^{-1})	0.73	2.16
K_{10} (h^{-1})	15.9	8.167
K_{12} (h^{-1})	0.032	0.532
CL (h^{-1})	0.006	0.083
V_{ss} (L)	0.195	0.103
C_{max} (μg/mL)	3.622	2.306

variation of 2.1% from the initial concentration was seen in ALOME, 2 years from the date of manufacture. The formulations were stable for 24 months under accelerated storage conditions of 25 ± 2 °C and 60 ± 5% RH. A maximum decrease of 3.5% from the initial concentration was observed Table 1. The average particle size was 11.3 μm and did not vary appreciably. More or less, it was spherical in nature and no physical changes like liquefaction or formation of lumps or discoloration were observed in the microspheres formulations during this 24-month study period; however, the changes were found to

Table 1
Observations of stability test studies in accelerated storage condition (25 ± 2 °C and 60 ± 5% RH).

Drug content (%)						Particle size (μm)						Physical change					
Months						Months						Months					
0	3	6	9	12	24	0	3	6	9	12	24	0	3	6	9	12	24
99.34	98.56	97.86	96.52	95.85	95.85	11.3	11.4	11.3	11.4	11.4	11.4	–	–	–	–	–	–

–: No physical change.

Table 3
Lung-targeting parameters of ALOME: ofloxacin after i.v.. administration in thirty number of albino mice. Data are presented as means ± SD ($n = 3$).

Sample	AUC		1	2	3	4	5
	ALOME	Ofloxacin injection	r_e	t_e	T_e	T_e^*	$T_e^* \times 100$
Blood	37.34	133.70	0.28	41.51	37.34		
Liver	50.05	144.35	0.53	20.42	75.90		
Spleen	26.54	9.73	1.84	58.42	26.90	0.91709	91.70
Lung	934.01	114.65	13.52	–	1550.18		
				$\sum T_e^* = 1690.32$			

be negligible and had no impact on the quality of the formulations. Fig. 5 shows decrease of drug content of ALOME under accelerated storage conditions.

3.5. In vivo pharmacokinetics and compartmental model studies

The in vivo pharmacokinetics of microspheres was studied using WINNONLIN Version 1.5 (Pharsight Corporation, USA) and was fitted by one-compartment model, two-compartment model and three-compartment model, respectively. Based on the analysis of the models and parameters, it was concluded that the in vivo pharmacokinetics of microspheres in blood could be best described by two-compartment model with intravenous injection (i.v.) (Fig. 6). The plasma concentration and pharmacokinetic parameters are reported in Fig. 7 and Table 2, respectively. From Table 2, one can see that in comparison with ofloxacin injection, ALOME altered the distribution of ofloxacin in vivo and the half-life after intravenous injection of ALOME ($t_{1/2(\alpha)} = 0.95$ h, $t_{1/2(\beta)} = 21.9$ h) were higher remarkably than those ($t_{1/2(\alpha)} = 0.37$ h, $t_{1/2(\beta)} = 10.7$ h) after intravenous injection of ofloxacin. The result showed that ALOME had sustained release efficacy.

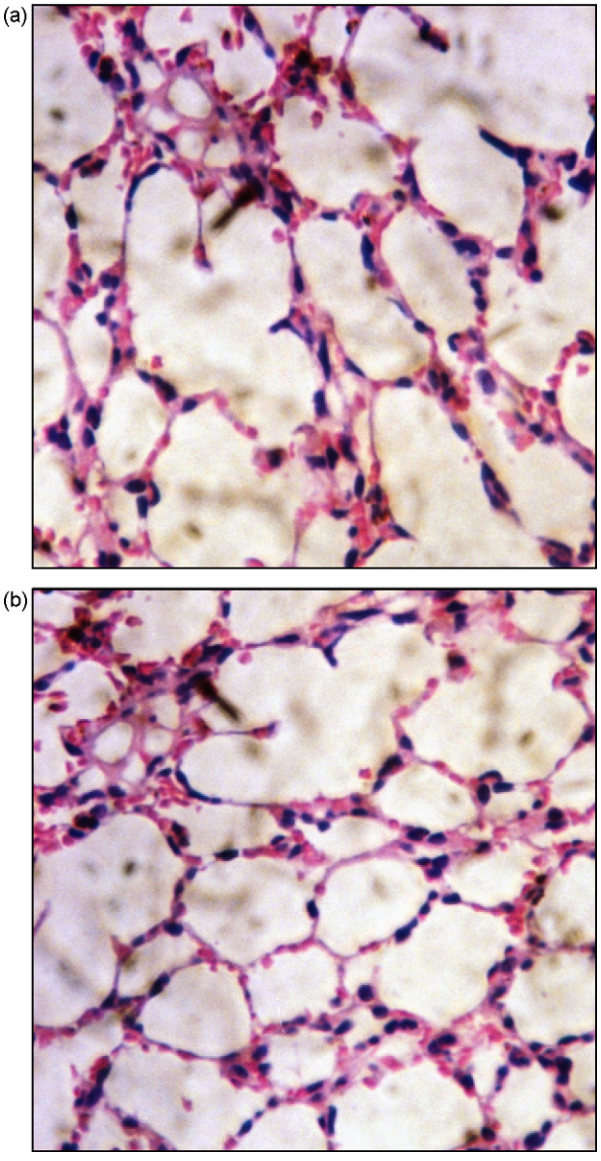

Fig. 8. (a) Cytoarchitecture of lung (control) and (b) cytoarchitecture of lungs after administration of ALOME.

The drug concentration in tissues indicated that the microspheres could deliver ofloxacin mainly to lung after intravenous injection to albino mice and the concentration of ofloxacin in lung (1048 μg g^{-1} after 15 min) was significantly more than that in any other tissues and blood. In comparison with ofloxacin injection, the drug concentration of ofloxacin in lung after intravenous injection of ALOME was 432 μg g^{-1} after 15 min.

Simple mathematical relationships have been suggested which may be useful in gaining a better appreciation of the in vivo performance of targeted drug delivery systems. The data of drug content with time were used to calculate the targeting parameters as shown in Table 3. ALOME showed the largest value of AUC and r_e for the lung; the targeting efficacy t_e of lung increased by a factor of 35 compared with spleen, ~18.5 compared with liver, ~25 compared with lung. Table 3, compares the time-averaged drug distribution to different tissues, following the intravenous administration of ofloxacin as a solution and albumin ofloxacin microspheres. All these, strongly indicated very significant lung-targeting characteristics of ALOME. Drug distribution to the lungs was 91.7 ± 0.3%.

In case of targeted drug delivery system such as microspheres, major portion of the drug and excipient are accumulated in specific tissue (lung) and therefore, determining the compatibility between these tissues and the microspheres formulation becomes a necessity in order to rectify the safety of the formulations. On comparison of the microspheres formulations with placebo microspheres, the cytoarchitecture of the tissue (lung) did not show any major difference (degenerative changes) as evidenced in Fig. 8a and b. Based on this observation, the constituents of the microsphere formulation were declared safe for parenteral use as a passive targeted drug delivery system to lungs.

4. Conclusion

The major conclusion of this work is that microspheres constitutes a useful means of passive targeting of drugs to the target organ and can be employed for effective anti-bacterial therapy. The findings of the work can also be applied for developing effective blue-print for targeted organ specific drug delivery; and the corollary of this work can be used as intracellular therapy with other drugs, by a slight modification in the techniques employed in the preparation of microspheres.

The prepared microspheres were found to possess suitable physico-chemical properties and the particle size range. The microspheres were found to release the drug to a maximum extent in the

target tissue. This work has provided an addition to the already significant domain to targeted drug delivery systems, which holds a promising alternative over the conventional means with which the most difficult to treat infections such as pneumonia can be managed effectively.

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