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ESTIMATION OF *INVIVO* PHARMACOKINETIC BIODISTRIBUTION DATAS IN ISONIAZID SLN NANOPARTICLE TARGETING LUNGS

***[1]K. Shahul Hameed Maraicar and [2]Thirumoorthy Narayanan**

[1]Professor, Department of Pharmaceutics, E.G.S.Pillay College of Pharmacy, Nagapattinam - 611 002, Tamil Nadu, India.
[2]Principal, Cherraan's College of Pharmacy, Coimbatore, Tamilnadu, India.

ABSTRACT

Solid lipid nanoparticles containing Isoniazid was prepared by using solvent evaporation method and *invivo* biodistribution of SLN in albino rat was evaluated to confirm the targetability of SLN in lungs. In this study it was investigated that a controlled release Solid Lipid Nanoparticle formulation containing Isoniazid was optimized by particle size with the help of different concentration of polymer and surfactant. To the best formulation *invivo* biodistribution studies were performed to prove the drug targeting in the lungs by SLN Nanoparticle. *Invivo* Biodistribution studies which was carried out for the best formulation shows that, Isoniazid Solid lipid nanoparticle accumulates maximum dose of Isoniazid in the lungs than other organs over prolonged period of time by enhanced Cmax and delaying the Tmax, which confirmed that inhalable SLN are suitable for targeting and providing sustained release of anti-tubercular drugs to lungs. So Inhalation is a selected route of administration for Isoniazid SLN.

Key Words:- Pharmacokinetic studies, Inhalation, SLN, Nanoparticle, Isoniazid.

INTRODUCTION

Solid lipid nanoparticles typically are spherical with average diameters between 50 to 500 nm. Solid lipid nanoparticles have a solid lipid core matrix that can solubilize lipophilic molecules. In which lipid core is stabilized by surfactants. SLNs offer exclusive properties such as smaller size, larger surface area, interaction of phases at the interfaces, and these are attractive for their ability to improve performance of neutraceuticals, pharmaceuticals and other materials. SLN are more stable than liposomes in biological systems due to their relatively rigid core consisting of hydrophobic lipids that are solid at room and body temperatures, surrounded by a monolayer of phospholipids. These aggregates are further stabilized by the inclusion of high levels of surfactants (Shah CV *et al*., 2011).

In order to overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by a solid lipid, which eventually transformed into solid lipid nanoparticles. The reasons for the increasing interest in lipid based system are many – fold and include, Lipids enhance oral bioavailability and reduce plasma profile variability, better characterization of lipoid excipients, an improved ability to address the key issues of technology transfer and manufacture scale up.

Solid lipid nanoparticles are one of the novel potential colloidal carrier systems as alternative materials to polymers, is identical to oil in water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has been replaced by a solid lipid shown on Fig. 1. They have many advantages such as good biocompatibility, low

Corresponding Author

K. Shahul Hameed Maraicar
Email: kemisha2002@yahoo.co.in

toxicity and lipophilic drugs are better delivered by solid lipid nanoparticles and the system is physically stable.

Solid lipid nanoparticles (SLNs) are considered to be the most effective lipid based colloidal carriers, introduced in early nineties. This is the one of the most popular approaches to improve the oral bioavailability of the poorly water soluble drugs (Cavalli R *et al*., 1996; Rishi Paliwal *et al*., 2009; Sailaja AK *et al*., 2011).

Nanostructured lipid carriers (NLC)

A new generation of NLCs consisting of a lipid matrix with a special nanostructure has been developed. This nanostructure improves drug loading and firmly incorporates the drug during storage. These NLCs can be produced by high pressure homogenization and the process can be modified to yield lipid particle dispersions with solid contents from 30–80%.Carrier system. However, the NLC system minimizes or avoids some potential problems associated with SLN.

1. Payload for a number of drugs too low
2. Drug expulsion during storage of formulations
3. High water content of formulated SLN dispersions.

The NLC are produced successfully by the high pressure homogenization method and it is possible to obtain particle dispersions with a solid content of 50 or 60%. The particle dispersions thus produced have a high consistency with a cream-like or almost solid appearance. NLC were introduced to overcome the potential difficulties with SLNs. The goal to develop NLC was to increase the drug loading and to prevent drug expulsion. This could be seen in three ways. In the first model, spatially different lipids composed of different fatty acids are mixed which leads to larger distances between the fatty acid chains of the glycerides and general imperfections in the crystal.

The highest drug load could be achieved and maintained by mixing solid lipids with small amounts of liquid lipids (oils) which are called imperfect type NLC. Drugs which show higher solubility in oils than in solid lipids can be dissolved in the oil and yet be protected from degradation by the surrounding solid lipids which are called as multiple types NLC, and are analogous to w/o/w emulsions since it is an oil-in solid lipid-in-water dispersion. Because of their properties and advantages, NLC may find extensive application in topical drug delivery, oral and parenteral administration of cosmetic and pharmaceutical actives.

The NLCs have been investigated in the topical and dermatological preparations, in the delivery of clotrimazole, ketoconazole and other antifungal imidazoles. The NLCs were also prepared to investigate whether the duration of brain targeting and accumulation of drugs in the brain can be enhanced by intravenous delivery. Apomorphine as a model drug has been targeted, through certain vessels, to selected brain regions by in vivo real-time bioluminescence imaging of the rat brain (Muller RH *et al*., 2002; Dong Y *et al*., 2009; Annette Zur Muhlen *et al*., 1998).

Lipid drug conjugates (LDC)

LDC nanoparticles can be termed as a special form of nanoparticles consisting of 100% LDC or a mixture of LDC with suitable lipids. Only highly potent low dose hydrophilic drugs may be suitably incorporated in the solid lipid matrix. In order to overcome this problem, the so called LDC nanoparticles with improved drug loading capacities have been developed. An insoluble drug-lipid conjugate bulk is first prepared either by covalent linking or by salt formation. The obtained LDC is then processed with an aqueous surfactant solution to a nanoparticle formulation using high pressure homogenization technique. Such matrices may have potential application in brain targeting of hydrophilic drugs in serious protozoal infections (Muller RH *et al*., 2002; Dong Y *et al*., 2009; Cavalli R *et al*., 2000; Cavalli R *et al*., 2003; Chen DB *et al*., 2001).

TUBERCULOSIS

Pulmonary drug delivery is attractive for both local and systemic drug delivery as a non-invasive route that provides a large surface area, thin epithelial barrier, high blood flow and the avoidance of first-pass metabolism. Tuberculosis (TB) is a disease caused by bacteria that are spread from person to person through the air. TB usually affects the lungs, but it can also affect other parts of the body, such as the brain, the kidneys, or the spine. In most cases, TB is treatable and curable; however, persons with TB can die if they do not get proper treatment. Multidrug-resistant TB (MDR TB) is caused by an organism that is resistant to at least Isoniazid and Rifampin, the two most potent TB drugs. These drugs are used to treat all persons with TB disease.

TB is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extra pulmonary TB). The disease is spread in the air when people who are sick with pulmonary TB expel bacteria, for example by coughing. In general, a relatively small proportion of people infected with *Mycobacterium tuberculosis* will develop TB disease; however, the probability of developing TB is much higher among people infected with the human immune deficiency virus (HIV). TB is also more common among men than women, and affects mostly adults in the economically productive

age groups. Without treatment, mortality rates are high. In studies of the natural history of the disease among sputum smear-positive and HIV-negative cases of pulmonary TB, around 70% died within 10 years; among culture-positive (but smear-negative) cases, 20% died within 10 years Tuberculosis (TB) has been a leading cause of death since time immemorial and it continues to cause immense human misery even today.

Tuberculosis is chronic granulomatous disease in major health problem in developing countries about 1/3rd of world population infected by mycobacterium tuberculosis it is apprehended with that unless urgent action is taken less than 15 million people worldwide including 4 million in India will die from tuberculosis in 1st decade of 21st century according to WHO as the ICMR 2001 estimate 40% adults in India are effected nearly 2 million people develops active disease every year about 0.5 million people die from it.

The World Health Organization (WHO) *Global Tuberculosis Report 2012* provides the latest information and analysis about the tuberculosis (TB) epidemic and progress in TB care and control at global, regional and country levels. It is based primarily on data reported by WHO's Member States in annual rounds of global TB data collection. In 2012, 182 Member States and a total of 204 countries and territories that collectively have more than 99% of the world's TB cases reported data.

Mycobacteria cause tuberculosis, Mycobacterium avium complex (MAC) disease, and leprosy. Tuberculosis remains the leading worldwide cause of death due to infectious disease. "First-line" agents for the chemotherapy of tuberculosis combine the greatest efficacy with an acceptable degree of toxicity, and the large majority of patients with tuberculosis are treated successfully with these drugs. Occasionally, it may be necessary to resort to "second-line" drugs.

Scaling up TB-HIV collaboration

Globally, 40% of TB patients had a documented HIV test result and 79% of those living with HIV were provided with co-trimoxazole preventive therapy in 2011. Interventions to detect TB promptly and to prevent TB among people living with HIV, that are usually the responsibility of HIV programmes and general primary health-care services, include regular screening for TB and isoniazid preventive therapy (IPT) for those without active TB. The number of people in HIV care who were screened for TB increased 39% (2.3 million to 3.2 million) between 2010 and 2011. Nearly half a million people without active TB were provided with IPT, more than double the number started in 2010 and mostly the result of progress in South Africa.

MATERIALS AND METHODS

Materials used: The active ingredients and excipients used for the formulation of SLN formulations are in laboratory standards. Isoniazid was attained as a gift sample from Hetero laboratories Pvt. Ltd, Hyderabad. Cholesterol from Himedia, Mumbai. And other excipients and solvents used in the research work are based on analytical standards.

Animals Used in the study

Swiss Albino Rats (Male and Female) weighing 150 – 200 g were used for oral bioavailability studies. The selected animals were housed in acrylic cages in standard environmental conditions (20–25 ºC), fed with standard rodent diet and water *ad libitum*. Ethical committee clearance was obtained prior to the study from the Institutional Animal Ethics Committee (SVCP/IAEC/03-0052).

Methods

Preparation of SLN

Solvent Evaporation followed by Ultrasonication

Isoniazid, Cholesterol and Span 60 are dissolved in ethanol and kept for some time in bath sonicator .The aqueous medium is prepared by dissolving tween 80 in distilled water and kept for stirring in magnetic stirrer for 15 mins. Upon evaporation of the solvent, the lipid phase is slowly added into the aqueous phase under continuous stirring. The nanoparticles dispersion is formed in the aqueous medium. The solution was kept in probe sonicator for 20 mins .Now repeat the same experiment with same amount of solvent, span 60 by adding stearic acid. The formulas are shown in table 1 (Muller RH *et al*., 2002; Dong Y *et al*., 2009; Cavalli R *et al*., 2000; Cavalli R *et al*., 2003; Chen DB *et al*., 2001; Cavalli R *et al*., 1996; Rishi Paliwal *et al*., 2009; Sailaja AK *et al*., 2011).

***In-Vivo* Evaluation of Isoniazid Loaded SLNs Pharmacokinetic Studies**

Swiss Albino Rats (Male and Female) weighing 150 – 200 g were used for oral bioavailability studies. All the rats were fasted for 12h before the experiment but had free access to water. Pure drug and Isoniazide SLN were administered to 6 rats in each group (Male: Female; 1:1) by oral feeding tube at the dose of 2.4mg/m^2 of Isoniazid. Blood samples were collected via the caudal vein at 0, 0.25, 0.50, 0.75, 1, 1.25, 2, 4, 6, 8 and 12 hours after administration separately. Blood samples were placed into tubes containing 0.3ml of anticoagulant solution and centrifuged immediately. After centrifugation, the plasma obtained was stored at -20^{0}C until further analysis (Parmar B *et al*., 2011; Suresh G *et al*., 2007; Xiang QY *et al*.,

2007; Yapingi Li *et al*., 2009; Rui Yang *et al*., 2011; Sumeet Sood, 2013).

Quantification of Plasma Concentration

Isoniazid plasma concentration was determined by HPLC of C_{18}-column (Analytical technologies 2000, India) analysis using Analytical technologies software at 263 nm. A 200µl plasma sample was placed into a centrifuge tube and 200µl of methanol was added and shaken vigorously for 30s at room temperature. After centrifugation at 4000 rpm for 15 min, the supernatant was separated and analyzed. Calibration curves were prepared by linear regression analysis of the plot of the peak area against concentration of Isoniazid. The concentration of Isoniazid in plasma samples was determined from the area of chromatographic peak using the calibration curve.

Data Analysis

Total calculations were done by software winnonlin noncompartmental analysis program 6.3.0.39 core version 04 jun2007 Peak concentration (Cmax) and time of peak concentration (t_{max}) were obtained directly from the individual plasma-concentration time profiles. Half life ($t_{1/2}$) was calculated from the terminal slope of the plasma concentration-time curves after logarithmic transformation of the plasma concentration values and application of linear regression. The basic calculations are based on the area under the plasma concentration versus times curve (zero moment) and the first moment curve (AUMC). The AUC can be calculated as before using the trapezoidal rule. The first moment is calculated as concentration times time (Cp x t). The AUMC is the area under the (Cp x t) versus time curve. Both the $AUC_{0\rightarrow t}$ and $AUMC_{0\rightarrow t}$ were calculated using the trapezoidal method. The area under the curve (AUC) determines the bioavailability of the drug for the given same dose in the formulation.

Bio-Distribution Studies of SLN- Isoniazid

For in vivo pharmacokinetic studies, Male Wistar rats weighing 160–180 gm were used. The protocol was duly approved by the Institutional Animal Ethics Committee (IAEC).

Route of administration: Inhalation

A group of male Wistar rats (n=6) received a single dose of 100 mg of SLN by inhalational route. About 500 mg of drug loaded SLN were charged & aerosolized by 30 actuation/30 sec using an in house apparatus to obtain inhaled dose of 100mg/animal (here with the help of blower the formulation is administered). Before dosing the rats were trained for 30 days to accept restraint & application of an infant inhalation mask attached to our in-house apparatus.

Sample collection: Collection of blood

After inhalation, the pharmacokinetic study was done by collecting blood sample at different time intervals viz., before dosing, 10 min after dosing & then for 1, 2, 4, 8, 12, 24 hr time periods by tail incision.

After blood collection animals were administrated with thiopentone sodium (45mg/kg, i.p) for deep anesthesia, there thoracic cavity is opened and tissues of interest is collected e.g. Lungs, Liver, Kidney were excised bottled dry, weighed & kept in triple distilled water at -20-20ºC. The collected organs are sliced and homogenized at 6000 rpm for 20 min. The tissue fluids are collected and centrifuged at 4000 rpm for 10 min and finally the supernatant is collected and analyzed by HPLC.

Collection of broncho -alveolar lavage

After sacrifice, thoracic cavity is opened; lungs intact with trachea are excised. The trachea was cannulated & lungs were repeatedly lavaged with chilled PBS (containing 0.5 M EDTA) broncho alveolar fluids were pooled, centrifuged and macrophages obtained were counted and kept at -20ºC for further analysis.

Sample preparation for analysis

The blood samples were collected from tail incision (0.5ml) was taken in heparinised micro-centrifuge tubes containing heparin equivalent to 50µl/ml of blood at different time intervals. Plasma was separated by centrifuging the blood samples at 4000 rpm for 10 min at 4ºC. Blood serum was collected and kept at -20ºC until analysis.

To 150 ml aliquots of plasma, 300 ml of de-protenizing agent (methanol) was added and the dispersion is vortexed for 2 min. The samples were centrifuged at 15,000 rpm for 10 min at 4ºC and supernatant is collected.

Isoniazid was extracted using 3 ml portions of chloroform-butanol (70:30 %v/v) and vortexed for 1 min followed by centrifuging at 4000 rpm for 10 min. supernatant was decanted , process was repeated for 3 times & supernatants was pooled. The collected supernatants were diluted and analyzed by HPLC.

Tissue sample preparation

20% (w/v) aqueous tissue homogenates were prepared in cold 150M KCl. The homogenates were centrifuged at 15,000 rpm for10 min at 4ºC and the clear supernatant thus obtained was used further. To 150µL aliquot of the clear tissue homogenates, 300µL of the methanol was added and the dispersion was

vortexed for 2 min. The samples were then centrifuged at 15,000 rpm for 10 min at 4ºC. The supernatant was collected and an equal volume of water was added. The samples were then filtered (0.20µm nylon filters) and were injected into the HPLC system.

Bio-analytical HPLC method

The collected serum samples were analysed by HPLC (Analytical technologies Ltd) comprising C-18 column & UV detector. The mobile phase consists of Triethanolamine acetate: acetonitrile (97: 3 %v/v) at 263 nm by isocratic elution method. The mobile phase was delivered at a flow rate of 0.9 ml/min and The injection volume was 20µL and the analysis was performed at 30˚C. A wash program which increased the % methanol was included at the end of Isoniazid elution to ensure washout of all interfering excipients. Spectral purity analysis of the Isoniazid peak over a range of 200–400 nm was performed. The accuracy and precision of the developed method for determination of Isoniazid was comparable to the isocratic methods described for Isoniazid in USP.

Data Analysis

The pharmacokinetic parameters were calculated based on a non-compartmental body model. The area under the concentration–time curve from time zero to time t (AUC0–t) was calculated using the trapezoidal method. Peak concentration (C_{max}) and time of peak concentration (T_{max}) were obtained directly from the individual plasma concentration–time profiles. The area under the total plasma concentration–time curve from time zero to infinity was calculated using Eq. as follows.

$$AUC_{0-\infty} = AUC0–t + Ct/Ke$$

where Ct is the Isoniazid concentration observed at last time and Ke is the apparent elimination rate constant obtained from the terminal slope of the individual plasma concentration. Time courses of serum drug concentration following inhalational route were analysed by winnonlin software program version 5.1 (pharsight corp NC).

BIO-DISTRIBUTION PHARMACOKINETICS STUDIES

The mean Biodistribution pharmacokinetic parameters of F3 SLN- isoniazid formulation administered through IV and Inhalation are summarized in the table 2 & 3, Fig 5-8. The C_{max}, T_{max} and Clearance of F3 formulation through Inhalation and IV was 36.82 µg/mL, 12hr, 1.2 and 18.24 µg/mL,12 hr,3.40 respectively.

By comparing the Cmax results between IV and Inhalation administration of Isoniazid SLN it shows that more concentration of drug was accumulated in the lungs while administering the formulation through Inhalation.

From the Tmax comparison data between IV and Inhalation administration it shows the sustainability time of Isoniazid SLN in lungs i.e., it confirms the sustained action of Isoniazid SLN in lungs.

By comparing the $AUC_{0-\alpha}$ form above tables 2-3, concludes that maximum concentration of drug was present in Lungs through inhalation than any other organ. And the organ clearance ratio of drug from lungs through inhalation was less than the IV administration, which confirms the sustained release of Isoniazid from SLN in the lungs.

Form the pharmacokinetic distribution data it shows that Isoniazid SLN shows more accumulation of drug in lungs through inhalation administration than IV, which indicates SLN is having targeted and controlled release of drug results in lungs.

By this it can be confirmed that inhalable SLN are suitable for targeting with negligible toxicity and providing sustained release of anti-tubercular drugs especially Isoniazid in lungs.

The results show the Isoniazid SLN leads to maximum deposition of drug in lungs through inhalation which leads to maintain high therapeutic concentration by improving good pulmonary tuberculosis chemotherapy.

Table 1. Composition of various formulation of Isoniazid SLN

Trial Formulation	F1	F2	F3	F4	F5	F6
Isoniazid (mg)	20	20	20	20	20	20
Cholesterol (mg)	200	200	200	-	-	-
Stearic acid (mg)	-	-	-	200	200	200
Span 60(mg)	100	100	100	100	100	100
Tween 80(ml)	0.5	1	1.5	0.5	1	1.5
Distilled Water(ml)	50	50	50	50	50	50
Ethanol(ml)	10	10	10	10	10	10
Sonication time	5 min	10 min	15 min	5 min	10 min	15 min

Table 2. Bio distribution studies of SLN- Isoniazid (F3) – IV Administration

Drug/route/origin	T_{max}(hr)	C_{max}(µg/ml)	AUC $_{0-\infty}$ (µg/ml/hr)	V_d (ml)	Clearance (ml/hr)
Lungs	2	18.24+3.4	848+54.4	244.80+8.26	3.40+ 0.04
Liver	3	24.98 +4.0	1006 +124.4	184.6+20.4	2.94+0.50
Kidney	5	22.02+2.42	680+20.90	218.4+12.6	3.22+0.46

Table 3. Bio distribution studies of SLN- Isoniazid (F3) – Inhalation Administration

Drug/route/origin	T_{max}(hr)	C_{max}(µg/ml)	AUC $_{0-\infty}$ (µg/ml/hr)	V_d(ml)	Clearance (ml/hr)
Lungs	12	36.82±4.2	1800±109.6	136.33±6.94	1.2±0.22
Liver	18	11.64±0.98	486±17.89	489±23.62	2.84±0.84
Kidney	22	5.24±0.10	218±11.29	756.6±88.5	2.34±0.52

*Standard deviation (n=3)

Figure 1. SEM Photography - Solid Lipid Nanoparticle (SLN)

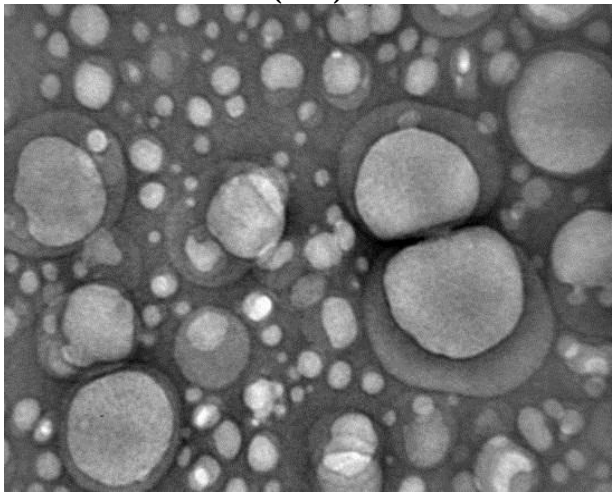


Figure 2. Types of NLC: (I) Imperfect type, (II) Amorphous type & (III) Multiple types.

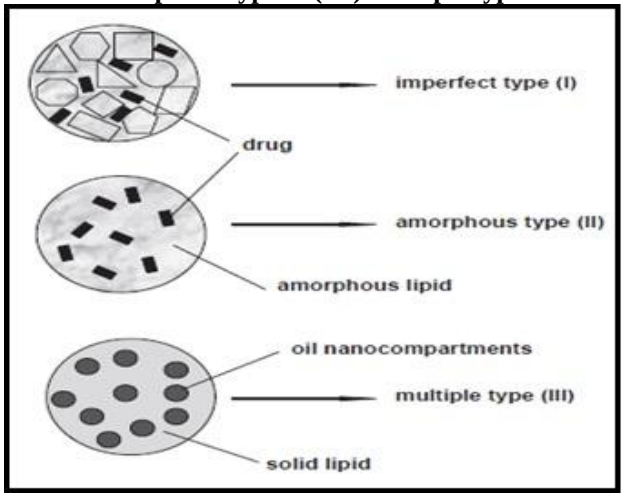


Figure 3. XY plot for Isoniazid SLN Nanoparticle –IV administration

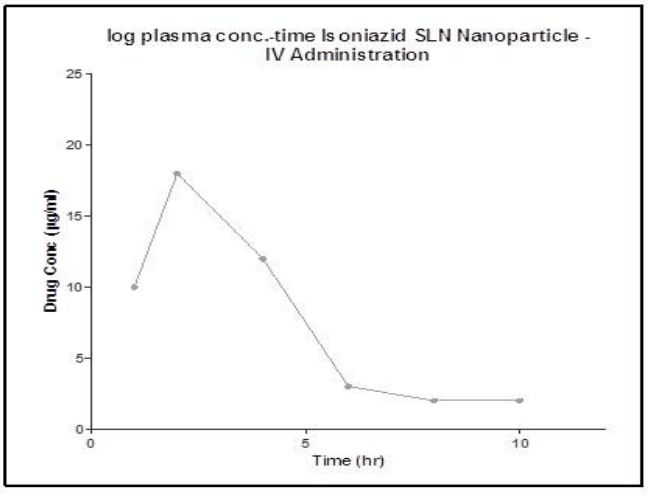


Figure 4. XY plot for Isoniazid SLN Nanoparticle – Inhalation administration

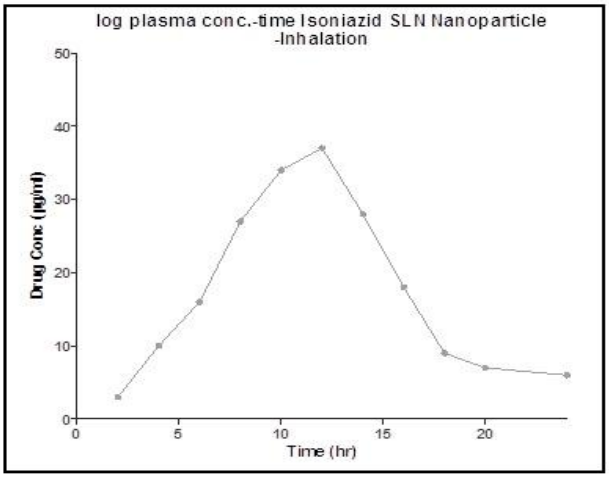


Figure 5. Comparison of Cmax concentration between Isoniazid SLN IV and Inhalation administration

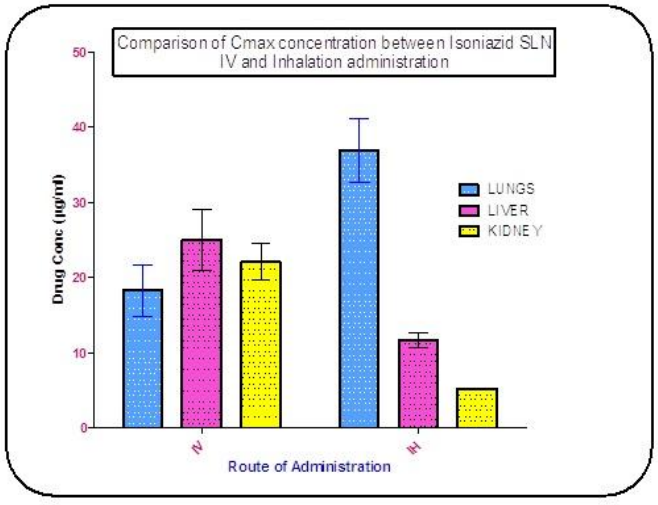


Figure 6. Comparison of Tmax between Isoniazid SLN IV and Inhalation administration in different organs

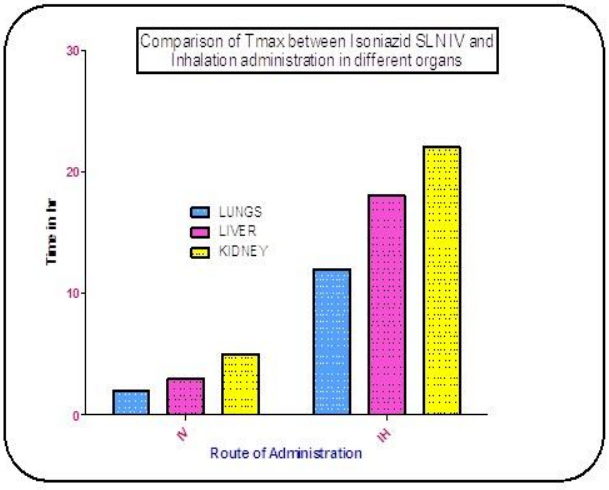


Figure 7. Comparison of Clearance ratio between Isoniazid SLN in IV and Inhalation administration in different organs

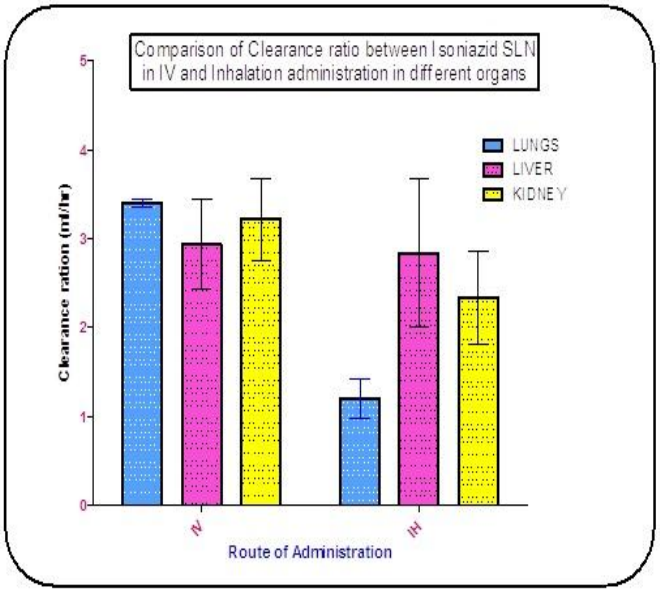
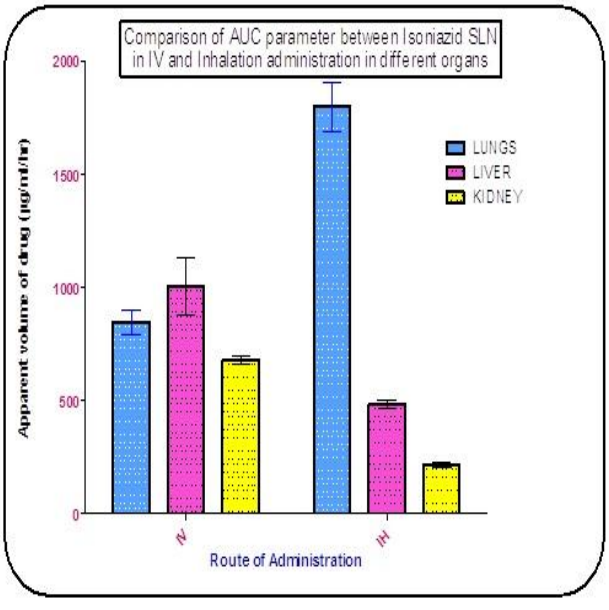


Figure 8. Comparison of AUC parameter between Isoniazid SLN in IV and Inhalation administration in different organs



CONCLUSION

From the pharmacokinetic biodistribution studies it was concluded that Isoniazid SLN nanoparticle type of dosage form was a selected carriers which was ease to target the Isoniazid drug, will leads to effective control of Lung Tuberculosis with less dose, dosage regimen and enhanced bioavailable dose due to targetability and controlled drug delivery of isoniazid from dosage form.

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