IN VIVO STUDY OF 5 FLUOROURACIL LOADED SLN (SOLID-LIQUID NANOPARTICLE) HYDROGEL

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Abstract

The main aim of the study to investigate the in vitro study of 5 Fluorouracil loaded SLN (solid lipid nanoparticle) hydrogel. For fulfill the study different type of lipids and excipients are used. 5 Fluorouracil was incorporated into the SLNs. SLN formulation is been characterized by zeta potential analysis, DSC etc. selected SLNs was loaded in hydrogel. The in-vivo study was determined for the administration of different types of doses. After, scarifying animal parts were taken out (liver, spleen, heart, skin, lungs and kidney) for the comparison to control. Study which was done skin irritant, acute dermal toxicity, sub-acute dermal toxicity(21days) and plasma concentration-time profile in animal.

KEYWORDS: SLNs (solid lipid nanoparticle), hydrogel, in-vivo study

INTRODUCTION

There are various types of route of administration of drugs. Topical drug delivery system is one of them. This route is used for the management of pain, incontinence of urination and contraception. This formulation is also used for the treatment of cutaneous disorders like acne, psoriasis. This may penetrate the skin layer or mucous membrane. It gives therapeutic action on application site.¹²³ These preparations maybe used in the form ointments, gels, creams, lotions, spray solution, eye drop, nasal drop. These formulations must have physical and chemical stability and produces sufficient amount of therapeutic profile. Formulation was releasing the medicaments and deliver to the site of action.⁴⁵

SLNs may provide some special properties like small size, surface area is maximum, medication stacking is high, interface communication, enhance the execution of pharmaceutical compounds. These may comprise the solide biodegradable lipids. SLNs may have favourable circumstances and well maintain at down side of colloidal carriers. These preparations may be used in various form like oral, parenteral, dermal, etc.⁶⁷

These are the novel potential colloidal transport system. In which polymers are used to distinguish to oil and water emulsion. these have more biocompatibility; it has low danger. It has lipophilic medication by SLNs.⁸⁹
In past some decades, the technology may be developed very much. The particle size is range from micro to nano scale. Magnitude may help in the reduction of particle sizes. In biomedical applications nanomaterials are the best vehicles due to good bioavailability and biocompatibility. Many researchers have mainly focus on new discovery by using new methods.\textsuperscript{10,11} These are useful in controlling the ADME of body, toxicity level in body, immunogenicity, biorecognition and drug efficacy. These are known as NDDS (novel drug delivery system). Many polymers are combined with this method. Different types of approaches are been used for the development of nanoparticles and may use in a various type of diseases. This drug delivery system has very good results in chronic diseases. It should be well satisfied the biopharmaceuticals and pharmacological conditions. Nanotechnology may increase the capability of proteomics, genomics and bioinformatics of compounds and combinatorial the chemistry.\textsuperscript{12,13} It should be more enthusiastic and may express to discover, invent and novel approaches by new technology. The drugs which have poor bioavailability are been use this drug delivery system. In which the therapeutic effectiveness may be depend on pharmacokinetics and the route of administration. Solubility, crystallinity, toxicity, and HLB value are been monitored in pharmacokinetics. For the distribution and absorption, the biopharmaceutics, pharmacokinetic, route of administration, surface area and drug transportation is play most important role. Hydrophilic and lipophilic molecules based on HLB value. Lipophilic properties may have very poor solubility. Solid lipid nanoparticles may play most important role in lipophilic molecules. Now a day lipid-based nanoparticle is used due to the presence of lipophilic molecules. The molecules have good biocompatibility, it may cross blood brain barriers, variability in particle size, most attractive lipid-based delivery system.\textsuperscript{14,15,16}

2. MATERIAL AND METHODS

5 Fluorouracil was supplied as gift sample. All excipients (Compritol, Sodium taurocholate Glyceryl Monostearate, Hydroxypropyl cellulose etc.) are supplied by Sigma-Aldrich, New Delhi, India. All the other chemicals are analytical grade.

**Incorporation of SLNs into Hydrogel**

**Hydrogel base formulation**

Different type of Gelling agent (Chitosan & Carbopol 934P) was used in weighed with ratio 1.5%, 2%, and 2.5% based on literature review. Boil the fresh water and after that cool the water mix it with Sodium Carboxymethylcellulose which is a synthetic gelling agent.

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Lubricating agent is been worn with glycerine and in glass mortar wetting agent is lost equally. Benzalkonium chloride fluid was pouring into the mixture with looped inspirational till suspenseful clear and the homogeneous hydrogel produced.

**Characterization of SLNs loaded Hydrogel**

**Visual Appearance**
In hydrogel, clarity play most important role. All formulation was appraised for transparency by visible information opposed a magazine background

**pH**
pH meter is been use for the striking pH of 5 Fluorouracil loaded SLNs hydrogel containing different ratios of Chitosan and Carbopol 934P as gelling agent.

**Viscosity**
The hydrogel's viscosity is also one of the parameters to be assessed. The viscosity of the formulations should be such that they are comfortable for the patient to apply. Using a Brookfield viscometer, the formulation's viscosity was measured at 250°C (ambient temperature) and 340°C (nasal temperature) (DV-I Prime).

**Gelling Strength**
Texture profile analysis is a simple approach for determining the properties of a polymeric system. The trials were carried out at Digital Scientific Equipment in RK Puram, New Delhi, with a TA-XT2 Texture Analyser. The gels were placed in a regular beaker beneath the probe for the experiment. After that, the sample is immersed in an analytical probe. With a test-speed of 1.0 mm/s, the Texture Analyser was set to the 'gelling strength test' mode or compression mode. The acquisition rate was set to 50 points per second, with a trigger force of 5 g. All of the samples were taken with a 7.6 cm diameter aluminium probe. The experiment was conducted at room temperature [9]. Gel strength was calculated as the force necessary to pierce the gel in g. \(^{129}\)

**Sol-To-Gel Transition (T\(_{sol-gel}\))**
Sol-gel transitions are important in determining the efficacy of formulations. 2 ml sample was placed in a glass vial and stirred at 15 rpm with a heating setup on a magnetic stirrer. The
temperature was steadily raised at a pace of 10 degrees Celsius per minute. The temperature at which the magnetic bead's rotation came to a halt was recorded as the gelation temperature. This may help in the evolution of hydrogel and sensitive for the gelation of spite and the created spectacular hydrogel for dissolution.

**IN-VIVO STUDY**

Adult albino mice were used in the in-vivo tests. The Institutional Animal Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals have approved a protocol for animal experiments [Protocol no. LCDP/IAEC/2020-21/018]. All process of animal study was conducted and guidelines were committee by CPCSEA. Adult albino mice were weighed about 160 to 250g was used for this study. These albino rats were used for the visualization of skin penetration, acute dermal toxicity and sub-acute dermal toxicity. For skin irritation study rabbits were used. For this animal standard cage is been taken and maintained the temperature (12 hrs light and 12 hrs dark). During the study animals were free for the food access. Before testing these animals were acclimatized in the laboratory conditions for 5 to 7 days before testing.

**Visualization of Skin Penetration**

Adult Albino mice weigh around 150 to 250g of either sex. These animals were group into 6 groups of 4 mice each. Dorsal region of the animal was shaved or trimmed. SLNs gels loaded 5 Fluorouracil were applied on a marked area at a dorsal site of animals group I, group II, group III, group IV, group V and group VI for 24 hrs. Animals were sacrificed by cervical dislocation. Blotted paper is been use for the skin excised. It was wash twice or thrice with ethanol. By using Confocal Laser Scanning Microscope, the area is sectioned into the pieces and evaluated the depth of penetration.

**Acute Dermal Toxicity**

Adult Swiss albino mice weight around 150 to 250gm is use for acute dermal toxicity. This Protocol was prepared as per ARRIVE (Animal Research Reporting in In Vivo Experiments). Adult Swiss albino Mice were divided into six groups (five test group and one control group). Each group have 3 mice. Group I contain control. Before 24-hour mice skin was trimmed and shaved. 5-FU SLNs gel dose induce into 16 mg/kg body weight were applied on the shaved surface of mice group II, III, IV, V and VI. This substance is been contact with skin porous dressing and non-irritating tape (3M) for 24 hours. This study was started from day 0. For
first 30 minutes to 24 hours, these animals were continuously watched for any changes. The surplus formulation was removed after 24 hours. For 15 days these animals were observed for any change in mortality and weight. If these animals show any mortality the administration dose is considered as toxic dose. If any mice show mortality out of three then the same dose is been repeated for the confirmation of toxic effect. Higher dose is been use if these formulations show no mortality observed. In this study, 5 Fluorouracil loaded hydrogel Formulation with dose level 15mg/kg and 75mg/kg for a higher dose testing. Organ and tissue of animals with Dose group and control group is been use for histopathological examinations. After 15 days experimental animals were scarified and store the parts (cervical dislocation. Skin, heart, liver, lungs, kidney, and spleen) for histopathological examination. These parts were preserved into 10% Formalin. These are then washed with tap water and dehydrated with ethanol series. These are then immersed in xylene and embedded in paraffin wax. By using microtone paraffin blocks were used. Haematoxylin and eosin were used for the staining and examined under the microscope.

Sub-Acute Dermal Toxicity [Repeated Dose 21 days]

According to ARRIVE (Animal Research Reporting in In Vivo Experiments) Swiss albino mice was use for the toxicity dose with 15mg/kg, 35mg/kg and 75mg/kg for 22 days. Around Adult Swiss albino mice weighing 150 to 250g were divided into six groups. 3 males and 3 females per group. Mice were shaved and trimmed before 24 hours of experiment. 5-FU-SLN hydrogel were applied into the shaved area of mice. These mice are group for 6 day per week basis over a period of 21 days. Weight of the body and consumption of food were measured and weekly recorded. Porous gauze is been applied on the treated area by non-irritating tape. Daily physical conditions were monitored and recorded. For haematology study, these animals were scarifying and the blood samples were collected. Theses blood sample were further use for the analysis of SGOT, SGPT, ALP, total protein, albumin, globulin, blood urea, creatinine, serum bilirubin, triglyceride, cholesterol, glucose and for sodium and potassium measurements. For histopathological study of animal organ and tissues with high dose and control is been taken after scarified animal. After that these organs and tissues are been preserved into 10% formalin for 24 to 25 hours. Wash and dehydrated by ethanol, xylene immersed and at last rapped with paraffin wax. Microtome is use for the cutting of these paraffins blocks. Haematoxylin and eosin were used for staining and after that it was examined under the microscope.

Skin Irritation Study
Male Rabbits were used for the skin irritation study. These animals are weighing around 2.1 to 2.8kg and use for testing of acute dermal irritation. These animals then group into 2 groups with 3 rabbit each group. Before testing, on dorsal surface the skin on both the side on trunk were removed. Approximately 7cm² area is been clean and marked of each of the animals. For positive control 20% sodium lauryl sulphate (SLS) solution were used. Group I 20% w/v SLS solution. Group II 5 Fluorouracil with SLNs gel. 600mg of test formulation were applied on each group of animals. Gauze patch is use for the covering of treated aera with non-irritating tape. Control is been use in all the groups. One animal from each group were use. After 24 hours, these formulations were removed from the skin with help of distilled water.

RESULTS AND DISCUSSIONS

Visual appearance
Formulations were appearance as white semi-liquid at Different ratio of Chitosan and Carbopol 934P Ratios & SLN 1%.

pH
The pH was range from 6.0 to 7.0. This was use for the acidity and basicity of the formulation.

Viscosity
Brookfield Viscometer is been use for the Striking viscosity. This was administrating the partake adapter and spindle at 25±0.6°C. Chitosan congeal was ranged from 844.111±2.15 to 845.120±2.49. In Carbopol 934P congeal was ranged from 29200±2.48 to 29800±2.78.

Gelling Strength
Chitosan gelling strength was range from 310 ± 2.19 gm/cm² to 365± 2.49 gm/cm². Carbopol gel ranged from 352 ± 3.15 gm/cm² to 368 ± 2.18 gm/cm².
Figure 1: Gelling Properties

Table 1: Characterization of Hydrogel

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>pH</th>
<th>Gelling strength (gm/cm²)</th>
<th>Viscosity</th>
<th>Spreadability coefficient (gmcm/sec)</th>
<th>Drug content (%)</th>
<th>Gelling capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHGB1</td>
<td>5.8</td>
<td>310 ± 2.19</td>
<td>310 ± 2.19</td>
<td>29.18 ± 1.09</td>
<td>85</td>
<td>+</td>
</tr>
<tr>
<td>CHGB2</td>
<td>6.2</td>
<td>325 ± 2.86</td>
<td>325 ± 2.86</td>
<td>30.49 ± 1.58</td>
<td>84</td>
<td>++</td>
</tr>
<tr>
<td>CHGB3</td>
<td>6.4</td>
<td>365 ± 2.49</td>
<td>365 ± 2.49</td>
<td>35.17 ± 1.28</td>
<td>97</td>
<td>+++</td>
</tr>
<tr>
<td>CAGB1</td>
<td>5.7</td>
<td>365 ± 2.49</td>
<td>365 ± 2.49</td>
<td>20.54 ± 3.48</td>
<td>83</td>
<td>+</td>
</tr>
<tr>
<td>CAGB2</td>
<td>6.1</td>
<td>352 ± 3.15</td>
<td>352 ± 3.15</td>
<td>21.85 ± 3.68</td>
<td>82</td>
<td>++</td>
</tr>
<tr>
<td>CAGB3</td>
<td>6.4</td>
<td>368 ± 2.18</td>
<td>368 ± 2.18</td>
<td>35.6 ± 2.25</td>
<td>91</td>
<td>++</td>
</tr>
</tbody>
</table>

(+) gelation within 50-60 seconds dissolve rapidly, ++ 80 seconds and remains stable for 4 hours, +++ 80 seconds and remains stable for 8 hours.
CHGB3 Shows better result for Gelling strength, Spreadability, Drug Content and Gelling capacity and finally selected as optimized formulation for further in vivo study

**In Vivo Skin Penetration Visualization**

For Topical Formulation the bio-distribution of drugs are crucial into the skin layer. the thickness of the skin is 20µm. the molecules of skin penetration by drugs are 400µm. For *in-vitro* skin penetration studies 5 Fluorouracil with SLNs gels are used. Rat skin is been use or the treatment with SLNs gel and a marker lipophilic rhodamine was instead for use. In the tissues the fluorescent model pathway was use for localization and permeation pathway. This can be distributed without any cryo-fixing or embedding in tissues. By using SLN gels the rhodamine is been penetrated into the skin. SLN gel show effective against the rhodamine on the rat skin up to 110 to 30 µm. results shows that the skin penetration were improved due to SLN gel. The *In-vivo* study found that 5 Fluorouracil was goes much deeper into the skin layer by SLN gel. This may help for performance as topical dosage form.

![Figure 2. Photomicrograph of confocal laser scanning microscopy of rat skin after 24 hours with marker (Rhodamine)](image)

**Toxicity Study**

For formulation tolerability of skin and biosafety studies a single dose acute and repeated dose sub-acute dermal toxicity was done. For this present study various dose of 5 Fluorouracil with effective concentration were selected. Clinical dose of the range 10 to 75mg/kg/d were used toxicity study. Dose level were range from 20mg/kg, 35mg/kg and 70 mg/kg in fixed dose (acute dermal toxicity) and in repeated dose 21 days dermal toxicity 20mg/kg, 35mg/kg and 70mg/kg.
Acute Dermal Toxicity

In this study the animals were observed for their daily changes in their body weight, intake of water and food and their mortality. In administration of SLN gels at the concentration of dose 20mg/kg, 35mg/kg and 70 mg/kg there were no drug related mortality were observed. During the observation periods (15 days) there is no such changes is been seen in any physical conditions of animals. In administration of SLN gels there were no skin erythema and edema was observed 15 days. In several animals there were no toxicity were observed due the administration of 5 Fluorouracil. The toxicity of 5 Fluorouracil on tissues and organs (GI tract, liver, kidney, heart, blood cells, bone marrow, skin etc) also studied.

Figure 3. Section of Skin of Mice - Control

Figure 4. Section of Skin of Mice - Treated with 5 Fluorouracil loaded SLNs gel (Acute Toxicity study at 70mg/kg)

Figure 5. Section of Heart of Mice – Control
Figure 6. Section of heart of Mice - Treated with 5 Fluorouracil loaded SLNs gel (acute toxicity study at 70mg/kg)

Figure 7. Section of liver of mice - Control

Figure 8. Section of liver of Mice - Treated with 5 Fluorouracil loaded SLNs gel (acute toxicity study at 70mg/kg)

Figure 9. Section of Lungs of Mice - control
Figure 10. Section of lung of Mice - Treated with 5 Fluorouracil loaded SLNs gel (acute toxicity study at 70mg/kg)

Figure 11. Section of Kidney of Mice - Control

Figure 12. Section of kidney of Mice - Treated with 5 Fluorouracil loaded SLNs gel (acute toxicity study at 70mg/kg)

Figure 13. Section of Spleen of Mice - Control

Figure 14. SLN gel treated for Spleen section of mice (70mg/kg)
Study of Sub-Acute Dermal Toxicity (Repeated Dose 21 days)

Repeated dose of 5 Fluorouracil loaded SLN gel is been given to the animals for 21 days there were no changes is been observed at any time points and in any concentration of dose. There were no significant varies was observed by weekly feed consumptions by comparing control group. This was shows that long term treatment of formulation has no adverse effects. The haematological parameters of bone marrow toxicities by SLN gels were evaluated in the repeated dose toxicity. In this experiment all blood is been done which was collected from 0 days to 21 days. Blood samples like Ht (haematocrit), WBC (white blood cells), RBC (red blood cells), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration) were detected. Some serum biochemical parameters like SGOT, SGPT, glucose, ALP (alkaline phosphate), total protein, albumin, sodium and potassium, albumin, globulin, serum creatinine, triglyceride and cholesterol of treated animal and control animals were compared. High dose was use for the treatment groups and controlled was performed. For histopathological studies liver, kidney, spleen, skin, and heart were taken out after sacrificed the animal. There were no inflammations, no cell lysis and no lesions were seen in histological evaluation. Results were clearly shows that there were no irritation occurs by the use of 5 Fluorouracil loaded SLN gels and there were no toxicities is been occurs in high doses which is been given to the mice. These results show that the biocompatibility and safety were confirmed.

Figure 15. Section of Skin of Mice - Control

Figure 16. Section of skin of Mice - Treated with 5 Fluorouracil loaded SLNs gel (Sub-Acute Dermal toxicity at 70mg/kg)
Figure 17. Section of Heart of Mice – Control

Figure 18. Section of heart of Mice - Treated with 5 Fluorouracil loaded SLNs gel (Sub-Acute Dermal toxicity at 70mg/kg)

Figure 19. Control section of liver of mice

Figure 20. Section of liver of Mice - Treated with 5 Fluorouracil loaded SLNs gel (Sub-Acute Dermal toxicity at 70mg/kg)
Figure 21. Control section of lungs of mice

Figure 22. Section of lung of Mice - Treated with 5 Fluorouracil loaded SLNs gel (Sub-Acute Dermal toxicity at 70mg/kg)

Figure 23. Control section of kidney of mice

Figure 24. Section of kidney of Mice - Treated with 5 Fluorouracil loaded SLNs gel (Sub-Acute Dermal toxicity at 70mg/kg)
Figure 25. Section of Spleen of mice - Control

Figure 26. Section of spleen of Mice - Treated with 5 Fluorouracil loaded SLNs gel (Sub-Acute Dermal toxicity at 70mg/kg)

Table 2: Pharmacological Behaviour (Acute Dermal Toxicity) 5 Fluorouracil loaded SLN hydrogel

<table>
<thead>
<tr>
<th>Fluorouracil loaded SLNs gels</th>
<th>0</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>55.69±4.59</td>
<td>46.85±2.41</td>
</tr>
<tr>
<td>Haemoglobin concentration (g/dl)</td>
<td>14.58±2.58</td>
<td>12.59±1.29</td>
</tr>
<tr>
<td>Red Blood Cells</td>
<td>15.49±1.49</td>
<td>10.59±5.56</td>
</tr>
<tr>
<td>White Blood Cells</td>
<td>9.65±1.59</td>
<td>9.56±1.29</td>
</tr>
<tr>
<td>Platelets</td>
<td>2045±80.5</td>
<td>2153±60.3</td>
</tr>
<tr>
<td>Mean corpuscular volume</td>
<td>51.48±4.59</td>
<td>49.59±2.49</td>
</tr>
<tr>
<td>Mean corpuscular haemoglobin</td>
<td>19.48±2.69</td>
<td>18.52±2.39</td>
</tr>
</tbody>
</table>
Mean corpuscular haemoglobin concentration | 38.49±3.67 | 35.69±3.58 | 34.59±2.67 | 36.48±3.29

Lymphocytes (%) | 67.49±6.49 | 62.39±3.59 | 66.59±1.29 | 65.49±5.29

Monocytes (%) | 1.89±2.49 | 1.52±0.632 | 1.26±0.89 | 1.7±1.29

Eosinophils (%) | 3.48±1.85 | 3.59±1.59 | 1.57±0.29 | 1.69±0.69

Basophils (%) | 0.66±0.65 | 0.94±0.56 | 0.65±0.35 | 0.56±0.67

Neutrophils (%) | 18.58±3.48 | 19.25±3.26 | 19.45±2.69 | 18.59±1.29

Table 3. Biochemical parameter of mice with fluorouracil loaded SLNs gel

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Fluorouracil loaded SLN gel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>SGOT</td>
<td>55.48±3.59</td>
<td>55.15±4.59</td>
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<tr>
<td>SGPT</td>
<td>48.25±2.15</td>
<td>44.67±4.29</td>
</tr>
<tr>
<td>Alkaline phosphate</td>
<td>55.67±1.25</td>
<td>55.18±4.29</td>
</tr>
<tr>
<td>TP</td>
<td>6.45±0.48</td>
<td>6.48±0.25</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.15±0.28</td>
<td>3.49±0.25</td>
</tr>
<tr>
<td>Globulin</td>
<td>3.15±0.48</td>
<td>3.74±0.29</td>
</tr>
<tr>
<td>Blood urea</td>
<td>30.15±3.15</td>
<td>28.49±3.49</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.45±0.32</td>
<td>1.59±0.23</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.85±0.12</td>
<td>1.28±0.15</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>192.48±15.49</td>
<td>188.58±12.59</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>182.48±16.58</td>
<td>159.2±12.29</td>
</tr>
</tbody>
</table>

Table 3. Biochemical parameter of mice with fluorouracil loaded SLNs gel
**Skin Irritation Study**

There were no significance changes were seen when treated with 5 Fluorouracil loaded SLNs gels at any time. Formulations were treated with 20% SLS. This treatment was applied on rabbit skin. There were no erythema and no edema sign were shown in this study. 20% SLS moderated with PII (primary irritation index) 2.34 and 2.1 were used for the skin irritation after 24 hours. This was indicated the safety of 5 Fluorouracil loaded SLN gel for skin application.

![Graph showing time dependent inhibitory effect](image)

**SUMMARY AND CONCLUSION**

The present study shows the “Formulation and development of solid lipid nanoparticles (SLN) loaded hydrogel for skin disorder.” *In-vivo* study was done on Albino mice. The result of visual observations indicated the reduction in inflammation after treatment with 1% w/w 5 Fluorouracil SLNs loaded in hydrogel. No drug related mortality was observed on administration of SLNs gel at the concentration of dose 20mg/kg, 35mg/kg and 70 mg/kg in acute dermal toxicity study. In sub-acute dermal toxicity study, repeated dose of 5 Fluorouracil loaded SLN gel was given to the animals for 21 days. There was no change observed at any time and in any concentration of dose which proved that long term use of this formulation has no adverse effects. Results of skin irritation study on rabbit skin clearly showed that there was no irritation occurred by the use of 5 Fluorouracil loaded SLNs gel.
REFERENCES


