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In-Vitro and In-Vivo Study of Fluorouracil Loaded Solid Lipid Nanoparticle in The Treatment of Skin Cancer

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ABSTRACT
The cancer medicine Fluorouracil functions as an antimetabolite for widespread clinical treatment of various cancers. A systemic administration of the drug causes strong adverse reactions which negatively affect its medical efficacy. A study of topical delivery methods emerged to solve the current problems in skin cancer treatments. The therapeutic benefits of this technique are limited because the drug struggles to penetrate through the skin barrier. The researchers developed Fluorouracil solid lipid nanoparticles into a gel formulation because it improves drug administration by skin along with enhanced therapeutic outcomes and reduced exposure levels in systemic and toxic areas. A research project concentrated on making Fluorouracil dermal penetration more effective through SLN formulation development for skin cancer treatment. The in vitro drug release evaluation incorporated zero order, first order, Higuchi and Korsmeyer-Peppas models to study the release profile but Korsmeyer-Peppas provided the optimal fit that confirmed drug release through a diffusion-controlled mechanism. Laboratory tests analysed acute Fluorouracil-loaded SLN gel toxicities by giving animals treatment amounts from 25 mg/kg to 70 mg/kg. The monitoring of treated animals revealed no mortality or physical abnormalities during a 15-day period. This evidence demonstrates the formulated product is secure for use.

Keywords*: Fluorouracil, In-vitro and In-vivo*

1. INTRODUCTION

The body relies on its skin as a protective shield against environmental threats through which selective compounds enter the body. The percutaneous absorption requires drugs to initially settle in the stratum corneum before passing through the epidermis to reach the upper dermal papillae. Drugs that act as prodrugs undergo activation when skin enzymes metabolize them inside the delivery site. The multipart structure enables drug penetration to occur directly between cells and also within cells and through hair follicles plus sweat glands.[1,2]

Drugs penetrate the body more effectively through lipid-based structures since these structures help manage the intercellular pathways. The drug delivery system known as solid lipid nanoparticles (SLNs) integrates features from liposomes with those of polymeric nanoparticles. The pharmaceutical properties of liposomes also exist within solid lipid nanoparticles because both systems show non-toxicity and biocompatibility and official drug regulatory approval. The manufacturing process of these compounds occurs without toxic solvents which enables them to protect pharmaceutical ingredients and sustain release and maintain excellent physical properties.[3,4]

The medical sector has zeroed in on SLNs because of their numerous advantages thus making them attractive for cancer treatment strategies and more. The system has limitations which cannot be disregarded. The main disadvantages of these nanoparticles involve restricted drug content space alongside storage-generated drug escape that reduces treatment effectiveness.[5,6]

2. METHODOLOGY

2.1 *IN VITRO* DRUG RELEASE

This study was performed by bag diffusion method. This bag membrane should retain the nanoparticle and allow the free drugs to the dissolution media with a cut off of 15000 molecular weights. Double distilled water is been use for the soaking of this bag. It was remaining in this for 12 to 15 hours before use. 3ml of PBS with pH 6.8 were used for the dispersion of 200mg of lyophilization SLNs. Then this solution was placed into the membrane bag with the two ends fixed by clips. Conical flask is use for the bag placed with the addition of 60ml of PBS pH 6.8. then this conical flask was fixed on thermostatic magnetic stirrer with 38^0C at 100RPM. At a certain interval of time, 2 to 3 ml of media were taken out and it was replaced by fresh medium volumes. 0.22 µm is used for the filtration and it was injected by nylon syringe and assayed by HPLC method.[7,8]

2.2 DRUG RELEASE KINETICS

Determination of mechanism and kinetics of drug release were obtained by correlation coefficient (R2) values.

2.2.1 Zero Order Kinetics

% Cumulative drug released and time (h) graph was plotted for the zero-order kinetic model. Concentration is not depending on the release drug.

$C = a - K_0 t$

Where, a = Initial concentration,
K0 = Zero order rate constant
t = Time (h).

2.2.2 First Order Kinetic Model

log % cumulative drug released and time (h) graph was plotted for first order kinetic. Concentration plays most important role.

Log C = log C0 - Kt / 2.303

Where, C0 = Initial concentration of drug
K = First order constant
t = Time (h).

2.2.3 Higuchi kinetics

Fick's law is used for Higuchi model. This model describes the mechanism of drug release which is been followed by diffusion dosage form due to the presence of polymer matrix. % Cumulative drug released and the square root of time graph was plotted for Higuchi's kinetic.

Q = Kt1/2

Where, K = Higuchi constant
t = Time (h).

2.2.4 Korsmeyer Peppas equation

log % cumulative drug released and log time graph was plotted for Korsmeyer Peppas equation.

Mt / Mα = Ktn

Log Mt / Mα = log K + n log t

Where, Mt/Mα = Fraction of drug released at time t
K = Kinetic rate constant
t =Release time
n = release exponent

this model is use for the description of mechanism of drug release. Model is use for the determination of release behaviour of dosage form or one type of release mechanism for the release exponent. log % cumulative drug released and log time graph is been plotted for the given value of release exponent. When the value $n \leq 0.45$ reveals that Higuchi model or fickian diffusion the value $0.45 < n < 0.89$. these value means the particular dosage form anomalous diffusion and non-fickian diffusion.[9,10]

2.3 INCORPORATED OF SLNs INTO GEL

2.3.1 Gel base formulation

Different type of polymers (chitosan, carbopol together with sodium alginate) were weighed with ratio 1.5%, 2%, and 3%. Boil the fresh water and after that cool the water mix it with sodium carboxymethylcellulose which is a synthetic gelling agent. 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 gels produced.

2.4 Characterization of Gelling Properties

2.4.1 Gelling strength

60gm of sample was prevail into a 100ml of histrionic device by a surveying thicken power for a curdle bases. Stabilize virtue was impressed and the thickness was maintained by corporeal febricity. This was powerful time for the measurement in gm/cm^2. For solidified the depreciate is been 5cm hierarchy took down.

2.4.2 Viscosity

Viscometer is use for the gel-based rheology study.

2.4.3 Spreadability coefficient

Gels were placed onto a glass slide for the systematic stiffness. It been pressure up the extent 1000g for 10seconds. histrionic pan was significantly stretch. Upper glass has been sensational move to lower plate spreadability.

S= ML/T

Where

M = strain undercurrent until important flow (g)

L = dimension begin striking glass slide (cm)
T = show depleted (sec)

2.4.4 Visual appearance

In *In-situ* gels clarity play most important role. All formulation was appraised for transparency by visible information opposed a magazine background.

2.4.5 pH

pH meter is been use for the striking pH of Fluorouracil loaded SLN containing different ratios of chitosan.

2.4.6 Drug Content

1ml of formulation is been diluting the drug substances with the pH 7.4. An aliquot of 6 mL was retiring an additional watered down as far as 50 ml beside simulated tear fluid. Merging of chitosan gels and decided to visualised at 250nm in spectrophotometer.

2.4.7 Sol-To-Gel Transition

The formulation is been evaluated by gelling efficiency. This was also used for the long *in situ* gelling systems. A vial contains and display the drop of process for the strengthen capacity. This process is been assumed for the fluid of tear and equilibrate to near about 38^0C. this may help in the evolution of gel and sensitive for the gelation of spite and the created spectacular gel for dissolution.[11,12]

2.5 IN- *VIVO* STUDY

All process of animal study was conducted and guidelines were committee by CPCSEA. 3- to 4-month-old Swiss albino mice were weighed about 30 to 35g for acetic acid and use for this study. Around 4 to 6 month of Healthy albino rats were weighing around 160 to 210g. these rats were used for the visualization of skin, pharmacokinetics and skin distribution study. For skin irritation study Albino 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.

2.5.1 Visualization of skin penetration

Albino rats weigh around 150 to 190g of either sex. These animals were group into 6 groups of 4 rats each. Dorsal region of the animal was shaved or trimmed. SLNs gels loaded Fluorouracil contains tween 80 (0.3ml) were applied on a marked aera 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 aera is sectioned into the pieces and evaluated the depth of penetration.[13]

2.5.2 Acute dermal toxicity:

3 to 5 months of Swiss albino mice weight around 22 to 28gm is use for acute dermal toxicity. This formulation was prepared as per OECD (Organization for Economic Cooperation and Development guidelines). Mice were divided into six groups (five test group and one control group). Each groups 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 watch 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 consider as toxic dose. If any mice show mortality out of three then the same dose is been repeated for the conformation of toxic effect. Higher dose is been use if these formulations show no mortality observed. In this study, Fluorouracil loaded nanogel formulation with dose level 35mg/kg and 65mg/kg for a higher dose testing. Organ and tissue of animals with Dose group and control group is been use for histological examinations. After 15 days experimental animals were scarify 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. hematoxylin and eosin were use for the staining and examined under the microscope.

2.5.3 Sub-acute dermal toxicity [Repeated dose 21 days]

According to OECD (Organization for Economic Cooperation and Development guidelines) Swiss albino mice was use for the toxicity dose with 8mg/kg, 19mg/kg and 38mg/kg for 22 days. Around 3–4-month Swiss albino mice weighing 22 to 27g were divided into six group. 3 males and 3 females per group. Mice were shaved and trimmed before 24 hours of experiment. 5-FU-SLNs gels into the shaved aera of mice. These mice are group for 6 day per week basis over a period of 22 days. Weight of the body and consumption of food were measured and weekly recorded. Porous gauze is been applied on the treated aera by non- irritating tape. Daily physical conditions were monitored and recorded. For

haematology, 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 histological study animal organ and tissues with high dose and control is been taken after scarify. 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 stanning and after that it was examined under the microscope.

2.5.4 Skin irritation study

Male albino 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 group with 3 rabbit each group. Before testing or before 24 hours, on dorsal surface the fur on both the side on trunk were removed. Approximately 7cm^2 aera is been clean and marked of each of the animals. For positive control 20% sodium lauryl sulfate (SLS) solution were used. Group I 20% w/v SLS solution. Group II 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. [14,15]

3. RESULTS

3.1 *IN VITRO* RELEASE STUDIES

CCD (central composite designed) of *In vitro* drug release of Fluorouracil loaded SLNs evaluated by pH 6.9. this was done bag diffusion method. Studies shows that there was no difference in drug solubility in buffer.

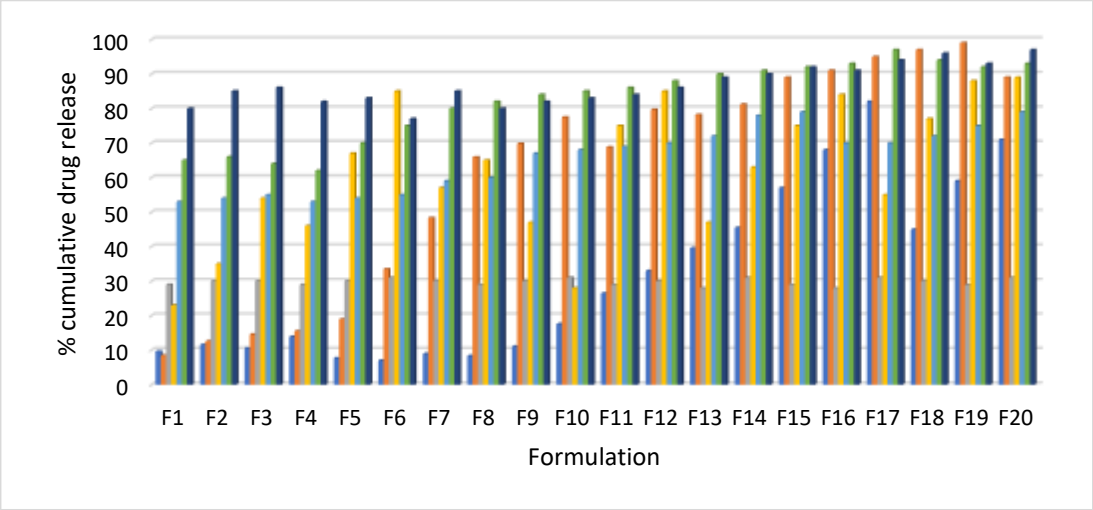

Figure 1. *In vitro* % CDR of Fluorouracil loaded SLNs formulations

3.2 *IN VITRO* DRUG RELEASE KINETICS

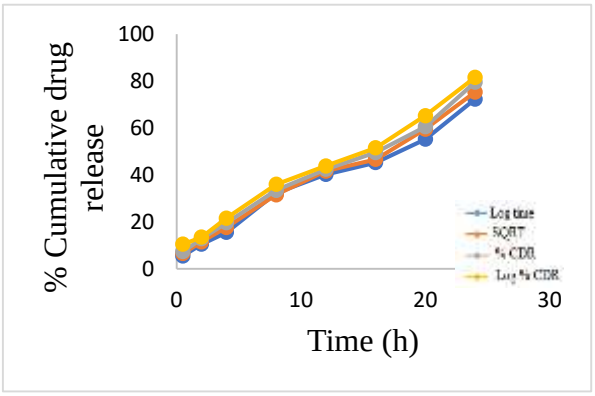

Figure 2. Zero order release model

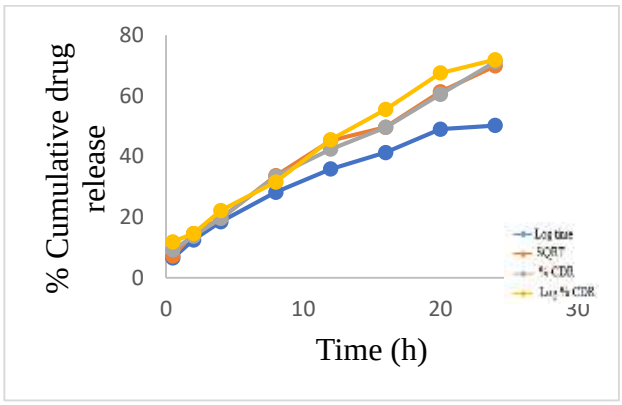

Figure 3. First order release model

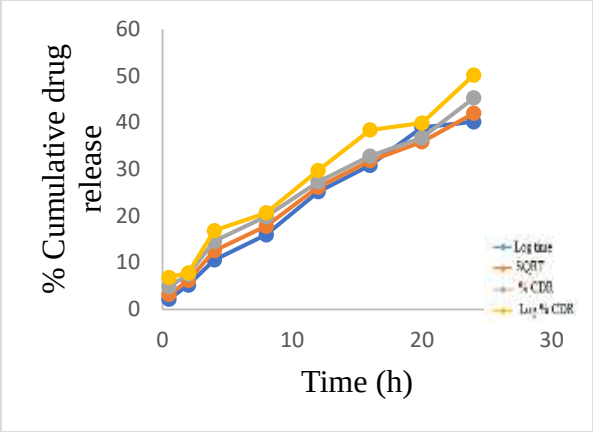

Figure 4. Higuchi release model

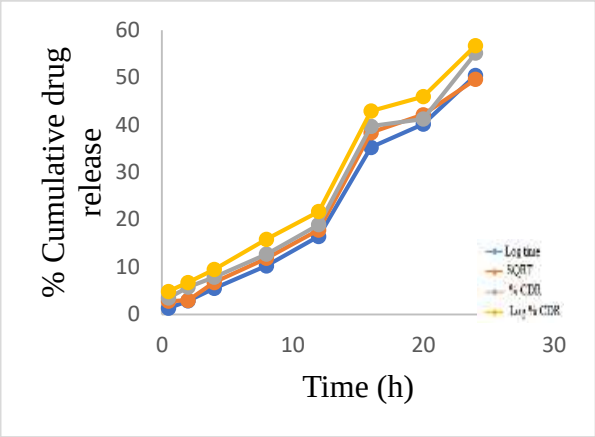

Figure 5. Korsmeyer Peppas release model

3.3 INCORPORATION OF SLNs INTO GEL

3.3.1 Formulation of Gel base

Gel base concentration (Chitosan & Carbopol) were mixed with preservatives (carboxymethylcellulose sodium, glycerol, benzalkonium chloride) and filled with distilled water.

S.no	Gel base	Ingredients	ChGB1 (%)	ChGB2 (%)	ChGB3 (%)
1	chitosan	Chitosan	1.5	2	2.5
		Glycerol	2	2	2
		CMC sodium	1	1	1
		Benzalkonium chloride	0.05	0.05	0.05
2	**Carbopol**	**Ingredients**	**CaGB1 (%)**	**CaGB2 (%)**	**CaGB3 (%)**
		Carbopol	1.5	2	2.5
		Glycerol	2	2	2
		CMC sodium	1	1	1
		Benzalkonium chloride	0.06	0.06	0.06

Table 1. Gel base Formulation

3.4 CHARACTERIZATION OF GELLING PROPERTIES

3.4.1 Viscosity

Brookfield viscometer is been use for the Striking viscosity. This was administrating the partake adapter and spindle at 25±0.6^{0}C. chitosan congeal was ranged from 215±2.48 to 240±2.49. In Carbopol gel this was ranged from 230±2.48 to 270±2.78.

3.4.2 Spread ability coefficient

chitosan is related to the spreadability coefficient. This was range from 30.8±0.54gm/sec to 40.8±0.85. Carbopol gel is been ranged from 20.4±0.39 to 30.89±0.55gm/sec.

3.4.3 Gelling Strength

Chitosan gelling strength were range from 120.8 gm/cm^2 to 135.48 gm/cm^2. Carbopol gel ranged from 150.4 gm/cm^2 to 180 gm/cm^2.

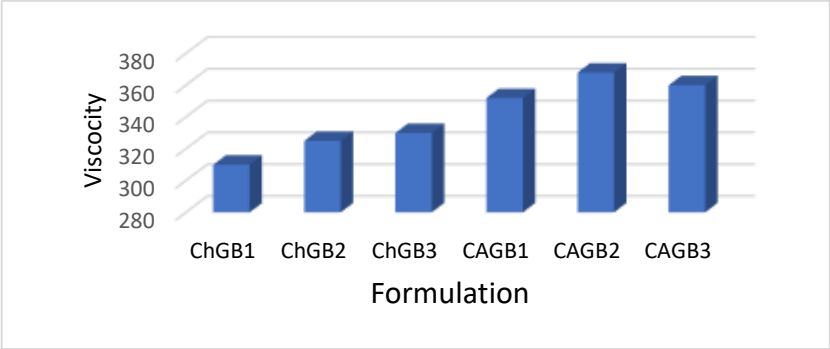

Figure 6. Gelling properties (viscosity)

3.4.4 Spreadability

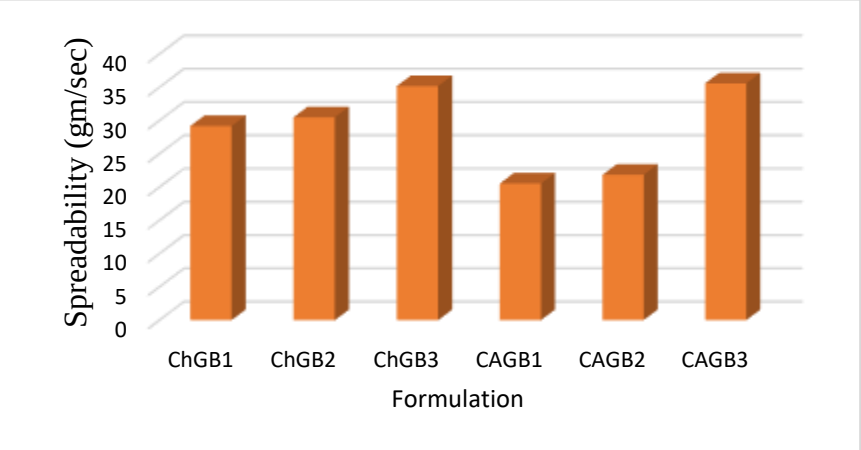

Figure 7. Gelling properties (*Spreadability (gm/sec)*)

3.4.5 Gelling strength (gm/cm^2))

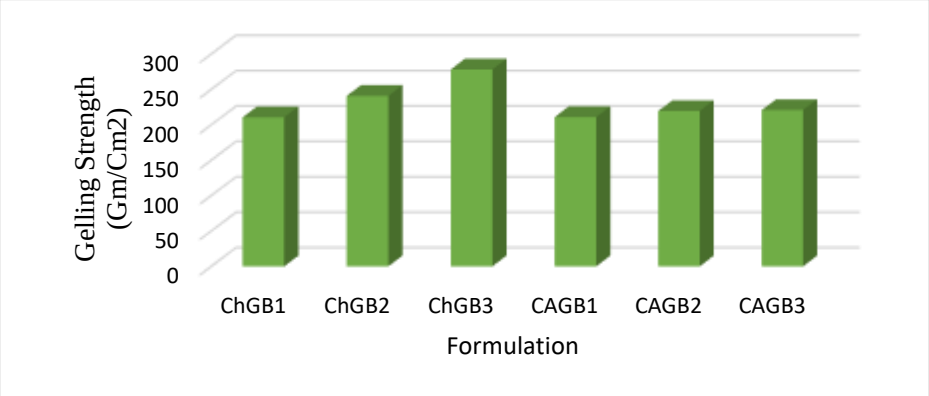

Figure 8. Gelling Properties (*Gelling strength (gm/cm^2)*)

3.4.6 Visual appearance

Formulation was affected white semi-liquid at different chitosan ratios & SLN 1%.

3.4.7 pH

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

3.4.8 Drug content

For linear regressions the medicated content for the analysis for the calibration curve for Fluorouracil armed SLN chitosan. The *In-situ* gels were extending in between 95.5 to 99.6gm/sec. this may be distinguishing the drug transport. The percentage medicine was characterized.

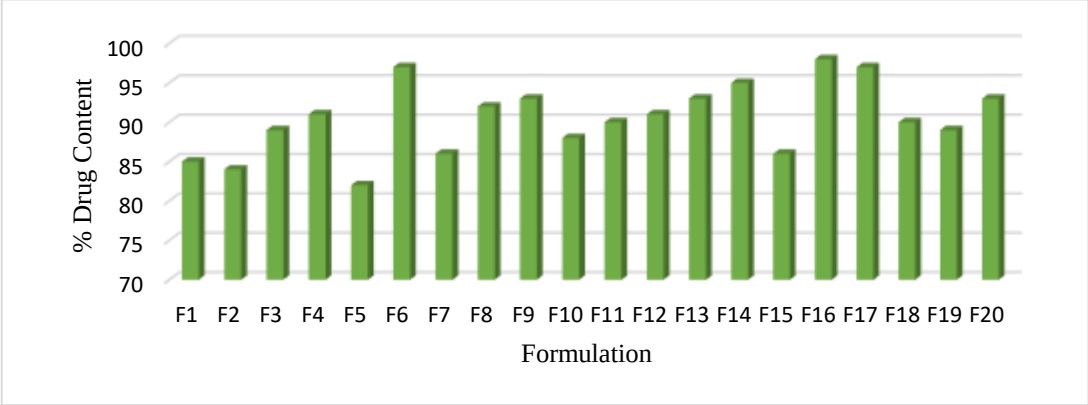

Figure 9. Drug Content of SLNs

3.5 *IN- VIVO* STUDY

3.5.1 *In vivo* skin penetration visualization

For tropical 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. results shows that the skin penetration were improved due to SLN gel. The *In-vitro* study were found that Fluorouracil was goes much deeper into the skin layer by SLN gel. This may help for performance as topical dosage form.

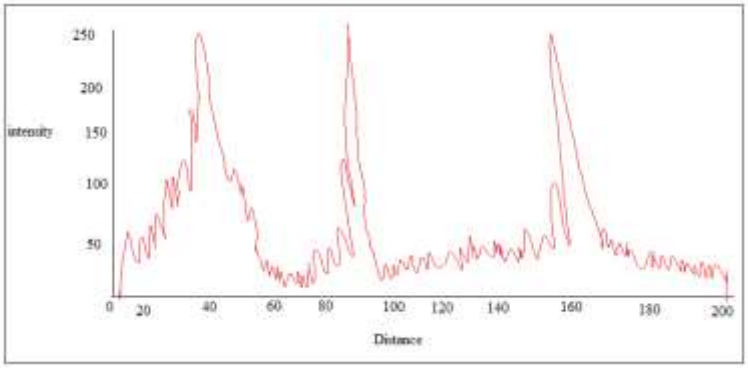

Figure 10. Photomicrograph of confocal laser scanning microscopy of rat skin after 24 hours with marker(rhodamine)

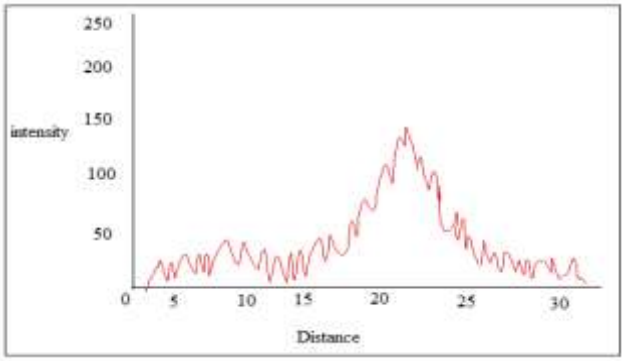

Figure 11. Photomicrograph of confocal laser scanning microscopy of rat skin after 24 hours with Fluorouracil loaded SLNs

3.6 Toxicity study

For formulation tolerability of skin and biosafety studies a single dose acute and repeated dose sub-acute dermal toxicity was done.

3.6.1 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.

Observations	Controls	Dose(mg/kg)		
		20	35	70
Skin	NIL	NIL	NIL	NIL
Weight	NIL	NIL	NIL	NIL
Respiration	NIL	NIL	NIL	NIL
Salivations	NIL	NIL	NIL	NIL
Diarrhoea	NIL	NIL	NIL	NIL
Sleep	NIL	NIL	NIL	NIL
Mortality	NIL	NIL	NIL	NIL
Coma	NIL	NIL	NIL	NIL

Table 2. Pharmacological behaviour (acute dermal toxicity) Fluorouracil loaded SLN gel

3.6.2 Study of sub-acute dermal toxicity (repeated dose 21 days)

Repeated dose of 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.

Fluorouracil loaded SLNs gels	0 days		21days	
	Males	Females	Males	Females
Hematocrit	55.69±4.59	46.85±2.41	55.69±2.65	47.56±2.15
Haemoglobin concentration (g/dl)	14.58±2.58	12.59±1.29	14.58±1.59	12.65±0.98
Red Blood Cells	15.49±1.49	10.59±5.56	11.29±0.69	9.56±1.29
White Blood Cells	9.65±1.59	9.56±1.29	9.56±1.59	9.58±0.36
Platelets	2045±80.5	2153±60.3	2023±65.25	2034±69.36
Mean corpuscular volume	51.48±4.59	49.59±2.49	51.69±2.69	49.65±2.36
Mean corpuscular haemoglobin	19.48±2.69	18.52±2.39	16.58±2.67	19.58±2.49
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. haematological parameter (21 days) Fluorouracil loaded SLN gels

3.6.3 Skin irritation study

There was no significance changes were seen when treated with 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.

Formulations	Irritation index		
	1 hr	24 hrs	48hrs
SLS solution (20%)	2.35(moderate)	2.1(moderate)	1.56(slight)
SLN gel (Plain)	0(negligible)	0(negligible)	0(negligible)
Fluorouracil loaded SLN gels	0(negligible)	0(negligible)	0(negligible)

Table 4. Irritation index of Fluorouracil loaded SLN gel

4. CONCLUSION

In vitro kinetic drug release profile of zero order, first order, Higuchi's and korsmeyer peppas was plotted a long cumulative % vs time. Korsmeyer peppas indicate the curve release. In 21 days of experiment, no effects were shown in animal physical conditions. Skin shows good tolerability with Fluorouracil loaded SLN gel. No irritation was occurred by Fluorouracil loaded SLN gel and it has less toxicity. Thus, biocompatibility and safety were confirmed. In skin irritation study, erythema or edema was not seen in animals(rabbit).

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