Original Article

Development And Characterization Of Antifungal Potential Of Topical Emulgel Formulation Of Tolnaftate

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Objective:

Abstract

Emulgels have emerged as a promising drug delivery system for the delivery of hydrophobic drugs. The objective of the study was to prepare emulgel of Tolnaftate. Intention of the current study is to expand nanoemulgel of tolnaftate as anti-fungal drug. The drug delivery system provides better-permeable delivery of effective moeighty via topical route in controlled and localized manner. Tolnaftate is most useful drug for take care of skin infections on human skin as athlete's foot, jock itch, and ringworm. It is an antifungal that works by preventing the growth of fungus. The nanoemulgels are characterize with a various number of parameters i.e. particle size, poly-dispersity index, drug content, spreadability, in-vitro permeation rate, microbial assay method. antifungal efficacy of develop nanoemulgels.

Keywords- Tolnaftate, Emulgels, Drug delivery system.

Introduction

Nanoemulsion is a variety of emulsion with drop dimension in the series of 10-250 nm. Nano-emulsion is including a variety of crucial carrier resources, i.e., polymeric nanoparticle and liposomes. The willingness of nanoemulsion with hydrogel skeleton is to deliver nanoemulgel exhibit more properties of thixotropic nature, non-oily, easily spreadable, effectively be expelled, emollient, not recoloring, and solvent in water, longer timeframe of realistic usability, bio-accommodating, translucent and pleasant appearance. Nanoemulgel contains different constituents and the idea of choice alongside assurance of wanted convergence of such segments include a practiced information in light of the fact that the properties of these are fluctuating from segments to segments, regardless of whether it is surfactant, co-surfactant, oil or potentially gelling specialist. The diverse assembling techniques have particular favorable circumstances influence the qualities of the last detailing. In this way, advancement of a thermodynamically steady nanoemulsion and nanoemulsion to nanoemulgel is exclusively relying upon reasonable choice of its parts and the essential strategy. Nanoemulgel dependent on hydrogel formulation with expansion of nanoemulsion converts into hydrogel lattice and can impacts a superior skin pore. This blend of nanomulgel has concerned the consideration of numerous researchers for the improvement of plenteous medications that capacity to treat different sorts of membrane problem.

Skin is the external tissue of the individual body. Persons are very responsive to exterior of their skin. Skin is the major appendage of individual as upper part having specific weight and surface area. Skin mainly covers 15 % area of the body weight and around 18, 000 cm^2 area of a mature person. The skin is the heaviest single organ in the body. Skin changes in thickness, shading, and surface. There are two significant kinds of skin: Thick and smooth, found on the palms and bottoms of feet in territories that are vigorously utilized (Stephen et al., 2010). The skin has three layers with various thickness, quality and capacity: Thin external layer is called Epidermis, Thick internal layer called Dermis, A greasy layer of subcutaneous tissue called Hypodermis or sub-cetaceous. Skin is a complex multi-layered tissue comprises of a scope of segments including veins, vessels, hairs, cells, filaments and so on. Skin has staggeringly complex structure that comprises of numerous parts. multi-layered structure of skin made by various layers of Cells, filaments and different segments and vessels, veins and nerves structure gigantic systems inside this structure. Also, hairs connect out from within skin, copious fine hair wrinkles are spread over the outside of skin. Skin executes a broad capacities coming about because of synthetic and physical responses inside these segments. Skin is a mind boggling organ framework perform numerous significant capacities, for example, go about as defensive obstruction against outside living beings, keep temperature control, detects our environment, annihilate squanders, and orchestrates Vitamin D. Skin likewise keep up the body in homeostasis (Stephen et al., 2010). Skin additionally stores fat and water, and assumes a job in invulnerability from sickness.

Oral route of administration is the most favoured route attaches in tolerant satisfaction, however, oral organization is increasingly inclined to hepatic first pass digestion required higher portion of medication (Kotta et al., 2014). Simultaneously the dispersion of medication all through the body can prompt mandatory symptoms. Henceforth the non-obtrusive, non-tormenting, non-aggravating topical conveyance of detailing is a substitute strategy related with a few points of interest, for example, conveyance of medication to explicit site of activity with decreased foundational danger, shirking of first pass digestion and gastric disturbance, expanding discharge pace of medication from definition to show signs of improvement percutaneous retention and for a minute topical application identified with increment bioavailability with supported discharge profile. Further its points of interest show, conventional transdermal plans, viz: balms, creams, moisturizers are in simultaneousness with numerous impediments, for example, clingy nature, absence of spreadibility, solidness issue, and so on., at last prompting understanding incompliance. Modernization of transdermal transportation is straight forward system by gel and emulgel with more prominent patient competence and better viability. In this manner, these details are securing interest both in beautifying agents' enterprises, just as in pharmaceutical ventures. Notwithstanding bunches of points of interest of gel and emulgel details, conveyance of hydrophobic medication despite everything stays a major impediment to traverse. Moreover, skin entrance through stratum corneum is likewise an extraordinary worry to the specialists for the fundamental action of the transdermal conveyance. The topical medication conveyance offers an immediate activity to the skin as an primarily organ part for determination and treatment unafraid of experiencing first pass absorption. Skin is solitary most promptly available organs on human structure for topical medication. Medications are directed topically for their activity at the site of use or proposed for foundational impact. The semisolid arrangements straightforward gels have extended both in beauty care products and in pharmaceutical arrangements. The advancement of measurements structures for topical medication conveyance is one encompassed by the many provoking territories to the plan researchers. Epidermis, dermis, and hypodermis are different layers of part of membrane. Stratum corneum of epidermis is the part of percutaneous medication via transport. The layer is comprised of dead cells of corneocytes, which need cores and organelles. Epithelial layer act as barrier of GIT, rectal, buccal, nasal and vaginal route. The greater part of new compound elements (around 40 % of the medications) at present being blended act as lipid loving layer in nature. This layer has wetting challenges and poor disintegration of materials for ultimately timely poor opening. These properties present a rate-restricting advance in their topical pervasion and successively cause an ensuing decrease in their viability. At the point when a medication is useful to the covering, regardless of whether inadequately or exceptionally dissolvable, just a little part of applied medication can enter from these topical measurements structures into fundamental dissemination. The significant bit of the medication stays in that capacity on the exterior part of the skin, this control their utilize on thin skin disease. The sickness contains infectious syndrome of the membrane, curls and on nail. These are predominantly named superficial mycosis effected by microbes Tinea

versicolor on membrane and hair. The disease called coetaneous mycosis as interdigital competitor's disease on foot. The restorative adequacy of topical opponent was effective by parasitic attack and treated by making a powerful medication. Topical affect a broad range of activities as restorative and dermatological on unhealthy skin. Topical medication conveyance framework has been utilized for quite a long time for the administration of neighborhood skin issue. Topical medication transport the drug and characterized as the use of a medication containing definition to the skin to treat cutaneous clutters like skin inflammation or psoriasis with goal of impact of medication to the exterior part of the skin or inside skin. There are two type of topical medication transport as, External topical and Internal topical. The topicals are applied to mucous film orally, vaginally or on rectal tissues for nearby movement. These details might be strong, semisolid to fluid. Medication substances are seldom controlled alone, however as a feature of a plan, in mix with at least one nonmedical operator that give assorted and concentrated pharmaceutical capacities. Medications having topical route for activity at the site of use have more impacts. Such medication can able to retain the drug entity through the skin by improved medication material with positive lipid/water segment coefficient and with nonelectrolyte. For the most part pharmaceutical arrangements useful to the membrane are utilized for some neighborhood activity and detailed to furnish delayed nearby contact with negligible fundamental medication ingestion. Prescription useful to the covering for enhance activity of materials to diseases include antifungal specialists, germicides, skin emollients, and protectants. Significant bit of leeway of topical conveyance framework is to sidestep first pass digestion. Other preferred position of topical arrangements are evasion of the dangers and bothers of intravenous treatment and of the shifted states of assimilation, similar to pH changes, nearness of compounds, gastric purging time. The topical medication conveyance framework is typically utilized where the others arrangement of medication organization not succeed or it is essentially utilized in contagious disease. Human skin is a huge and effectively open organ offers perfect and various destinations to control restorative operators for both neighborhood and fundamental activities. Human skin is a deeply capable for remove hindrance proposed internal parts and the outside of structure. Emulgel is emulsions, containing oil in water or water in oil type. All of these emulsions are gelled by blending in with a gelling specialist. A few antifungal specialists are accessible available in various topical arrangements (for example creams, treatments, and powders with the end goal of nearby dermatological treatment). One of these antifungal specialists is Itraconazole, which has both antifungal and antibacterial properties. It applied locally in gentle dermatophyte and cutaneous contaminations. The gellified emulsion was stable and enhanced medium for water insoluble medications. The oil in water and water in oil type of emulsions were converted to geling type structure in nature by blending with a gelling structure. Oil in water emulsions are usually supportive as water repulsive medication bases and use for healing purposes. The water in oil type of emulsions are utilized generally for the management of dry membrane and act as emollient applications. The topical medication conveyance framework diffuses sedate out of the conveyance framework ranges to the site of activity and get consumed by the skin. The discharge pace of the medications from topical planning is relying straightforwardly upon the physiochemical properties of the bearer and the medication utilized (Gungor et al., 2013.; Chen et al., 2011; Choudhury et al., 2017).

In the mid-1980's, Emulsion-gels have been achievement noteworthiness in pharmaceutical topical semisolid measurement structures. Emulgels are emulsions, might be oil-in-water or water-in-oil type, and gelled by blending in with a gelling specialist. The USP included that gels are semisolid formulations enclose any suspensions or inorganic particles, or massive natural particles interpenetrated by a liquid. Gel structures cross connected system, inwhich little medication particles catch and gives its discharge in a controlled way. As mucoadhesive property of framework, it drags out the contact time of medicine over the skin. As biphasic fluid dosages structures, emulsion is a controlled discharge framework where ensnared, tranquilize particles present in interior stage go through the outer stage to the skin and gradually get ingested. The inward stages go about as repository of medication, which gradually discharge sedate in a controlled route through the outer stage to the skin (Lachman, 2014).

Gels and emulsions have a significant restriction as their powerlessness to conveyance of hydrophobic medications and precariousness during capacity separately. So to defeat these restrictions an emulsion move towards i.e., Emulgel is being utilized so a hydrophobic restorative moiety is effectively fused and propelled one of a kind property of gels (Panwar et al., 2011, More et al., 2016). Emulgel has the property of both emulsion and gel it acts

indicated the double control discharge framework. Emulgel propose the capacity of conveying both hydrophilic and lipophilic medication moieties because of quality of both watery and non-fluid stages. It is logically useful to the covering because of its non-oily quality in evaluation to other topical details, for example, balms, creams and so forth. The utility of a few topical planning bolster its entrance ability and need to vanishing of materials or sleekness from skin. The course of use of infiltration into skin is simple, when emulsion is thixotropic; it turns out to be less thick during shearing. In this way, to improve emulsion dependability and entrance capacity and convert into gel. Conveyance of hydrophobic medications: The hydrophobic medications have greater dissolvability issues and can't be brought straightforwardly into gel base and hence issue emerges during the arrival of the medication. Emulgel hydrophobic medications are fused into the oil stage and afterward sleek globules are scattered in fluid stage bringing about o/w emulsion, emulsion can be all around blended into gel base. This might be giving better strength and arrival of medication (Vats et al., 2014).

Tolnaftate is an effective drug for tinea cruris and tinea corporis, and most cases respond in 1–3 weeks. Because of poor penetrability, it is less effective in tinea pedis and other hyperkeratinized lesions. For the same reason, it is ineffective in tinea capitis (involving scalp) and tinea unguium (involving nails). Symptomatic relief occurs early, but if applications are discontinued before the fungus bearing tissue is shed—relapses are common. Resistance does not occur. Salicylic acid can aid tolnaftate by keratolytic action. Tolnaftate causes little irritation, but is inferior in efficacy to imidazoles. It is not effective in candidiasis or other types of superficial mycosis. Tolnaftate is a synthetic molecule work of thiocarbamate derivatias act as anti-fungal agent or fungicidal or fungistatic property. Tolnaftate is a selective, reversible and non-competitive inhibitor of membrane-bound squalene-2,3- epoxidase, an enzyme involved in the biosynthesis of ergosterol. Inhibition leads to the accumulation of squalene and a deficiency in ergosterol, an essential component of fungal cell walls, thereby increasing membrane permeability, disrupting cellular organization and causing cell death. In adding, it alters the hyphae and aerobatics mycelial enlargement in susceptible fungi. Tolnaftate is a topical fungicide can prevent ergosterol biosynthesis by inhibiting squalene epoxidase. It also deform the hyphae and to stunt mycelial growth in liable organisms.

Method and Materials:

Material:

Tolnaftate was obtained from Sigma Aldrich, Crabomer 940, Polysorbate 80, Glycerine, Sodium Acetate, Edetate sodium was procured from Himedia Laboratories Pvt. Ltd., Benzyl alcohol was purchased from Plethico Pharmaceuticals Pvt. Ltd., Poly Vinyl Pyrollidone, Methanol, Methyl Paraben was purchased from Loba Chemie Pvt. Ltd., Mumbai. Chloroform and Diethyl ether was purchased from Rankem Ltd, New Delhi and S. D. Fine Chemical Limited, Mumbai.

Methods:

The study included the following steps.

Analytical Methods

Determination of absorption maxima (λmax): Absorbance of drug at various concentration range 10mg, 25mg, 40mg, 65mg and 80mg at pH 6.8 was taken by using UV spectrophotometer (Raymond et al., 2006, Jain et al., 2009).

Preformulation Studies

Microscopic examination: Microscopic examination of the plumbagin sample was done to study the nature / texture of the powder. A pinch of drug powder was spread on a glass slide and observed under phase contrast microscope.

Physical Characteristics

Density: The drug powder was exactly weighed (M) and poured gently through a glass funnel into graduated cylinder and the volume was noted and bulk density was determined. The tapped density was determined using tapped density apparatus.

Particle size: The average particle size (d_{avg}) of drug was determined by using a microscope (66172/Olympus, 100 X, Olympus (India) Pvt. Ltd., New Delhi) fitted with ocular micrometer and stage micrometer. The content of tolnaftate in each sample was determined by using UV-Visible spectrophotometric method.

Preparation of nanoemulsion

Tolnaftate material containing nanoemulsion was set up by effectively utilizing fast homogenization technique. The creation of medication and different substances**.** The composition of formulations was prepared by containing the changeability in sum and piece proportion of edetate disodium, glycerin and polysorbate 80 with 20 % w/w refined water (watery stage). Medication TFT was included the predefined amount of fluid paraffin (oil stage) till to scatter in nature. The nanoemulsion contains two stages known as fluid stage and slick stage. The nonstop stage was set up by dissolving sodium acetic acid derivation, disodium edate, glycerin and polysorbate 80 in purged water. The medication TFT was scattered in fluid paraffin known as scattered stage. The scattered stage was included into persistent stage by fast homogenization process with homogenizer blender with 2500 rpm. The homogenization process was subsequently increased to 5000 rpm for 1 h. It was carried out up to 15 min in same rpm as 5000 rpm. The pH of prepared mixer was adjusted to 6.8-7.0 (neutralizing medium) by using 2 N sodium hydroxide solutions by adjusted volume appropriately. The nanoemulsion (TFTNE) was obtained after high pressure homogenization method (Karri et al., 2015, Karri et al., 2018).

In-vitro Anti-fungal activity study

The result of in-vitro anti-fungal study of prepared formulation TNEG1 was perfromrmed to find out the ability of zone of inhibition on both the microorganism naming i.e. Tinea Pedis & Tinea Cruris.

In-vivo evaluation The albino Wistar rats (Male) of 150 ± 20 g weiged size were used for topical fungal cure studies. We collect 24 animals from house and divided into four groups containing 6 animals each. Group I animals were take care of marketed cream with tolnaftate drug as positive control, whereas Group II act with finalized optimized formulation nano-emul-gel TNEG1 respectively. The animals were maintained at a temperature of 21 ^{0}C and were allowed access to feed and water. T. Pedis was an infectious agent and used for the study of susceptibility to fungal infection than T. cerius. The micro-organism was obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. The obtained fungal species were maintained on Sabouraud Dextrose Agar (SDA) at 4^{o}C, and sub cultured once a month (Fujita 1997, 1992, Van Cutsem 1989).

The dorsal surface of each animal having an area of 5 cm^2 was depilated and applied 7 day old cultured infectious inoculum of T. Pedis on it and left for 3 days. The presence of scales and redness as initial symptom oneach animals on the 5th day was observed. Now the treatment was started on the 5th day post inoculation until complete recovery from the infection was achieved. The therapeutic efficacy of the nano-emul-gels was evaluated on daily basis by macroscopic examination of lesion (Srpska et al., 2007).

Stability studies

The nano-emulgel (TNEG1) are the semi-solid topical drug delivery system were prepared with various polymeric blend and oils at temperature 2±2°C, 25°C & 60% RH and 40°C & 75% RH, 50°C & 75% RH, 60°C & 75% RH for a period of 180 days.

Results and Discussion

Analytical Methods

Determination of absorption maxima (λmax): Absorbance of drug at various concentration range 10mg, 25mg, 40mg, 65mg and 80mg at pH 6.8 was taken by using UV spectrophotometer.

The spectrums are shown in Figure 1 to 6.

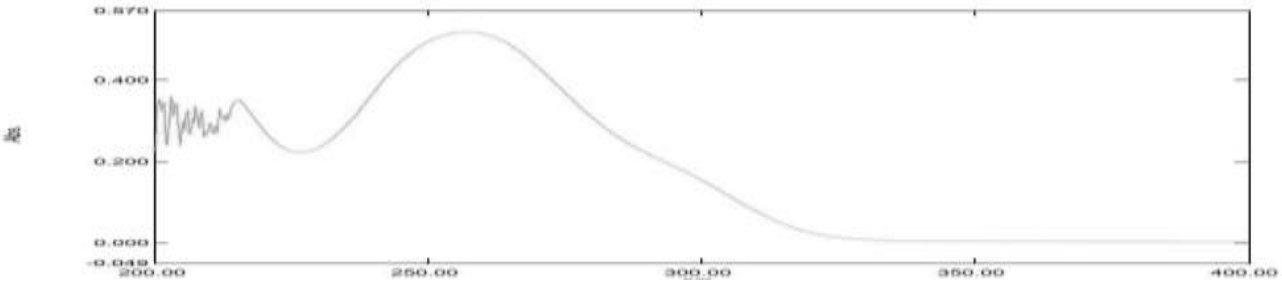


Figure 1: Maximum absorption (λ-max) of tolnaftate (10 mg) in phosphate buffer pH 6.8 solution

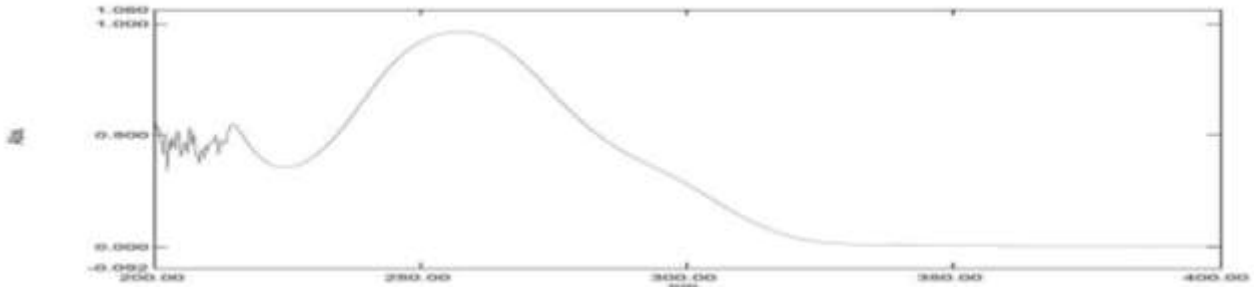


Figure 2: Maximum absorption (λ-max) of tolnaftate (25 mg) in phosphate buffer pH 6.8 solution

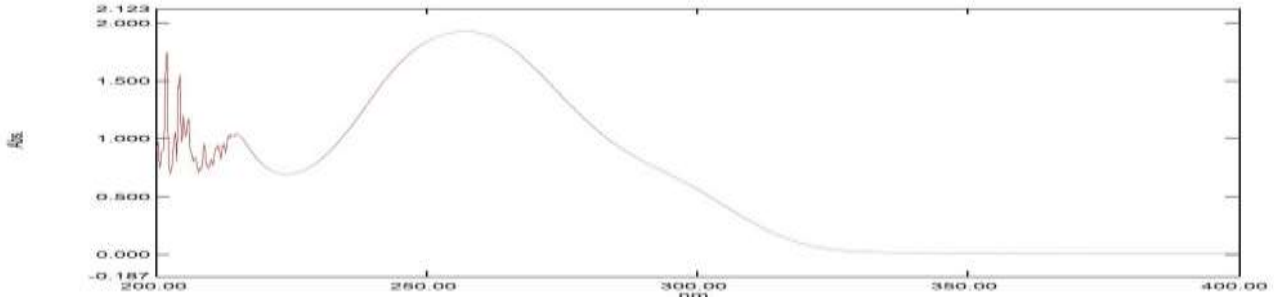


Figure 3: Maximum absorption (λ-max) of tolnaftate (40 mg) in phosphate buffer pH 6.8 solution

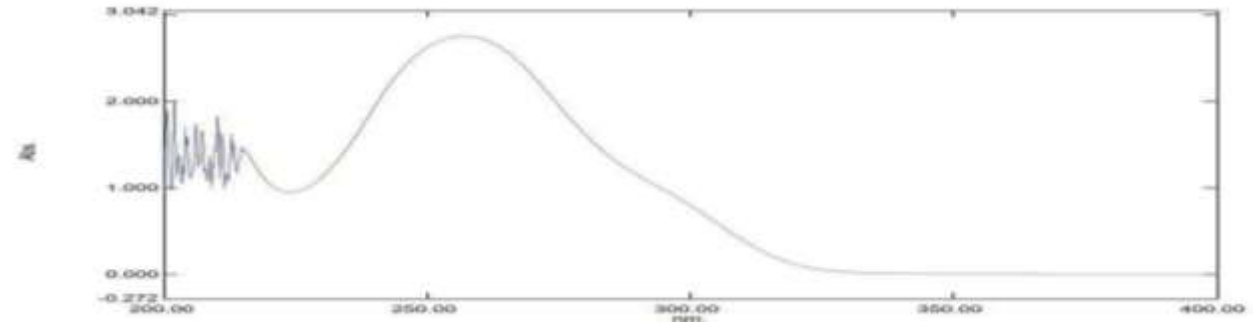


Figure 4: Maximum absorption (λ-max) of tolnaftate (65 mg) in phosphate buffer pH 6.8 solution

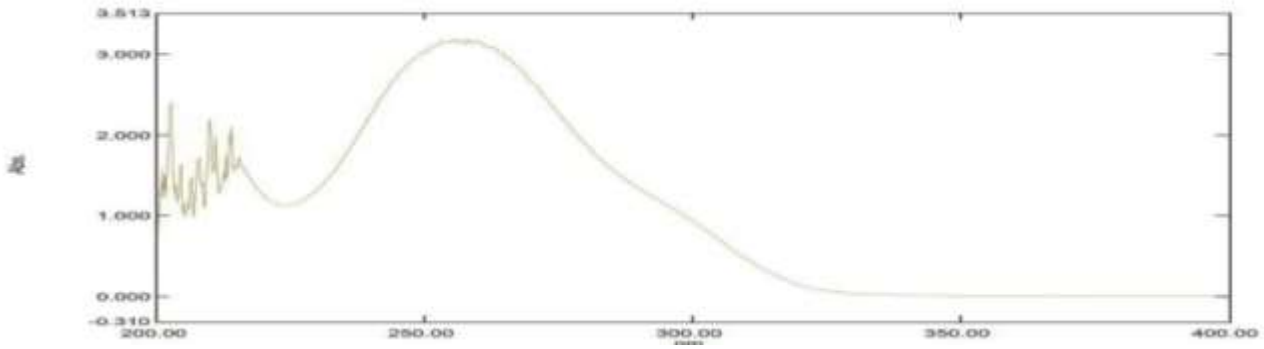


Figure 5: Maximum absorption (λ-max) of tolnaftate (80 mg) in phosphate buffer pH 6.8 solution

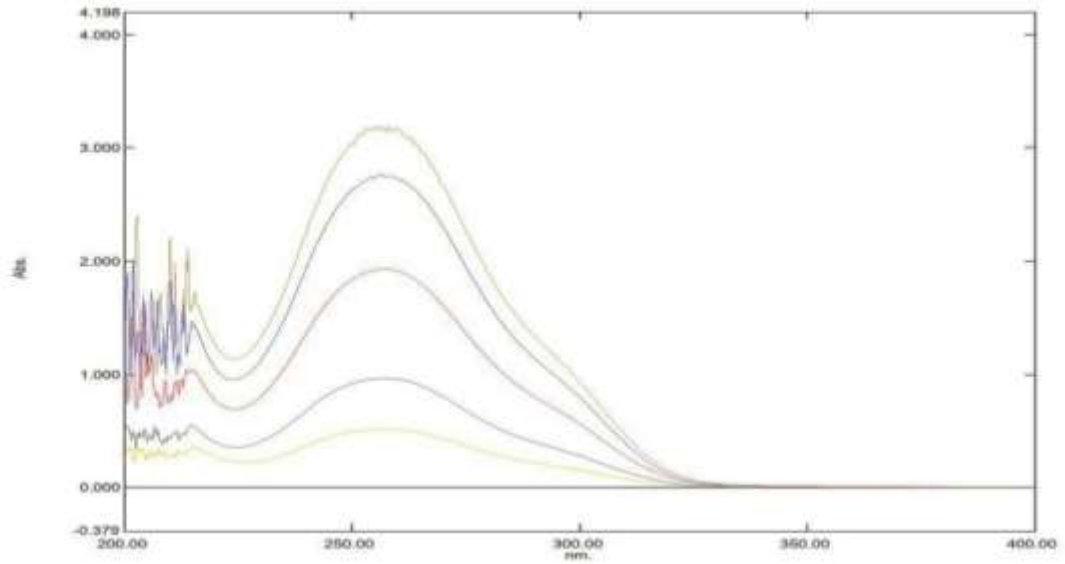


Figure 6: Overlay graph of maximum absorption (λ-max) of tolnaftate in phosphate buffer pH 6.8 solution

Preformulation Studies

Microscopic examination of the plumbagin sample was done to study the nature / texture of the powder. A pinch of drug powder was spread on a glass slide and observed under phase contrast microscope (Table 1).

Table 1: Organoleptic characteristics of Tolnaftate

Properties	**Tolnaftate**
Color	Whitish yellow
Odor	Slightly pungent
Taste	Slightly sweet

Figure 7: Microscopic examination of the plumbagin sample

Physical Characteristics

Density: The drug powder was exactly weighed (M) and poured gently through a glass funnel into graduated cylinder and the volume was noted and bulk density was determined. The tapped density was determined using tapped density apparatus. A bulk and tapped density of tolnaftate is to be 0.312 gm / cm^3 to 0.326 gm / cm^3, respectively.

Particle size: The average particle size (d_{avg}) of drug was determined by using a microscope (66172/Olympus, 100 X, Olympus (India) Pvt. Ltd., New Delhi) fitted with ocular micrometer and stage micrometer. The particle size of unmilled Tolnaftate powder was 41 μm.

Miscellaneous: The flow property, solubility and compatibility study of drug was performed and shown in Table 2, 3 and 4 respectively.

Table 2: Flow properties of drug

Drug	**Type of powder**	**Carr's index (%)[a]**	**Hausner's ratio [a]**	**Angle of repose θ [a]**
Tolnaftate	Unmilled	12.38±0.018	1.12±0.014	26.4±0.121
	Milled	9.06±0.016	1.03±0.007	18.9±0.093

Table 3: The solubility of tolnaftate at different pH medium (n=3)

Media	**Solubility (mg / ml)**	**Mean**	**% RSD**	**Standard error of Mean**	**Lower 95 % C I**	**P Value**
Water	0.00093	0.00097	0.000021	0.000011	0.0117	< 0.0001
0.1 N HCl	0.00156	0.00165	0.000037	0.000042	0.0144	< 0.0001
Phosphate buffer pH 4.5	0.00091	0.0096	0.000025	0.000011	0.0101	> 0.01
Phosphate buffer pH 6.8	0.00101	0.00102	0.000188	0.000053	0.011	> 0.01
Phosphate buffer pH 7.4	0.00094	0.00093	0.000104	0.000776	0.0270	< 0.0001

Table 4: Drug-excipient combinations for compatibility study

Batch no.	Drug-excipient combinations
S1	Pure drug tolnaftate
S2	Tolnaftate + all excipients

Figure 8: The I. R. Spectrum of sample of pure tolnaftate (S1)

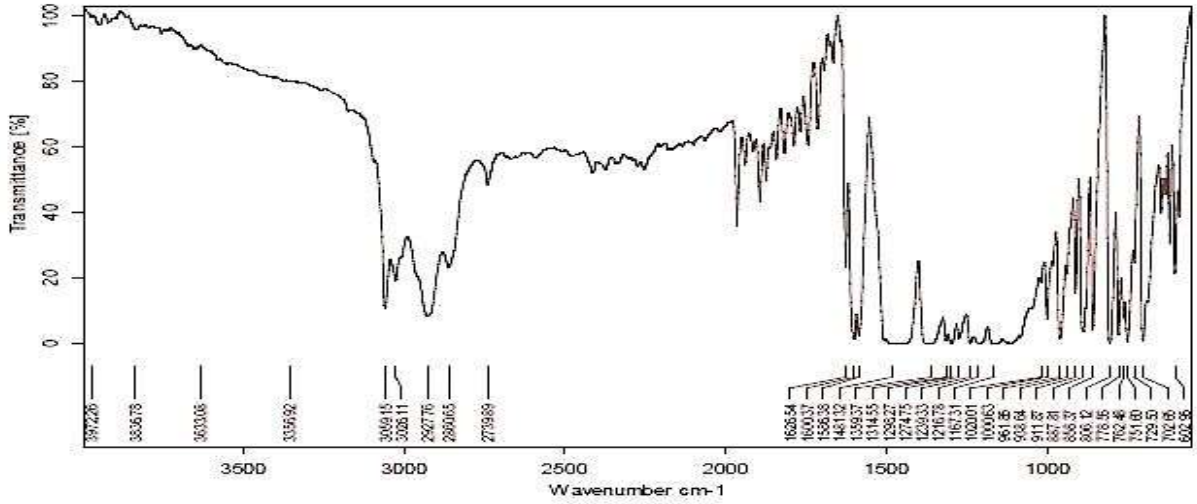
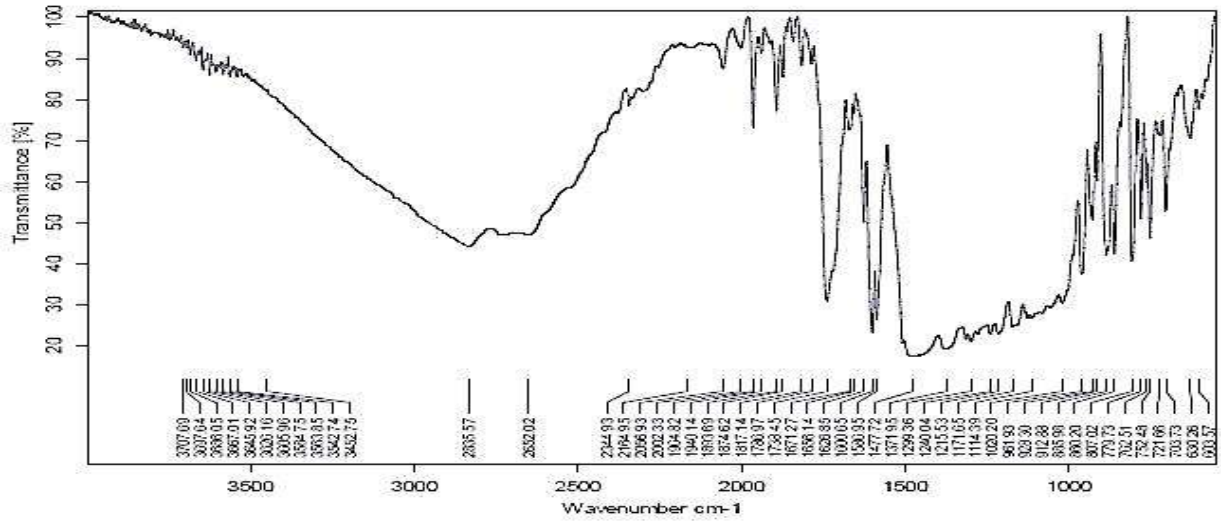


Figure 9: The I. R. Spectrum of sample of tolnaftate with excipients (S2)



Preparation of nanoemulsion

Tolnaftate material containing nanoemulsion was set up by effectively utilizing fast homogenization technique. The creation of medication and different substances to get ready different details is appeared in **Table 5-8.**

Table 5: Formulation of nanoemulsion

S. No.	Component	Components Utility	Amount (%Wt/Wt)

1	Drug	Active Pharmaceutical Ingredient	1.00%
2	Oil	Carrier	5.00%
3	Polysorbate 80	Oil in water Emulsifier	2.00%
4	Glycerine	Humectent	1.00%
5	Sodium acetat	Bufering agent	0.20%
6	Edetat disodium	Anti-oxidant	0.02%
7	Benzyl alcohol	Preservative	2.00%

Table 6: Formulation of nanoemulsion (Fixed oil (Neem oil)

F. Code	Drug (%)	Emulsifier (1 % v/v)		Oil (% v/v)	Water (ml)
		Surfactant (% v/v)	Co-surfactant (% v/v)		
		Polysorbate 80 (Tween 80)	PEG		
FN1	1	20	80	4	2
FN2	**1**	**40**	**60**	**4**	**2**
FN3	1	60	40	4	2
FN4	1	80	20	4	2
FN5	1	100	0	4	2

Table 7: Formulation of nanoemulsion Fixed oil (Flaxseed oil or Linseed oil)

F. Code	Drug (%)	Emulsifier (1 % v/v)		Oil (% v/v)	Water (ml)
		Surfactant (% v/v)	Co-surfactant (% v/v)		
		Polysorbate 80 (Tween 80)	PEG		
FL1	1	20	80	4	2
FL2	**1**	**40**	**60**	**4**	**2**
FL3	1	60	40	4	2
FL4	1	80	20	4	2
FL5	1	100	0	4	2

Table 8: Formulation of nanoemulsion Volatile oil (Tea tree oil)

F. Code	Drug (%)	Emulsifier (1 % v/v)		Oil (% v/v)	Water (ml)
		Surfactant (% v/v)	Co-surfactant (% v/v)		
		Polysorbate 80 (Tween 80)	PEG		
FT1	1	20	80	2	2
FT2	**1**	**40**	**60**	**2**	**2**
FT3	1	60	40	2	2
FT4	1	80	20	2	2
FT5	1	100	0	2	2

Evaluation of nanoemulsion

Nanoemulsions were discovered thermodynamically stable frameworks and were shaped at a specific grouping of oil, surfactant and water, with no stage partition, creaming or breaking. The parameter thermostability is significant one of parameter for separates nanoemulsion from emulsions. It was give the aftereffect of motor steadiness and in the long run stage independent. Consequently, the readied plans were utilized to various thermodynamic security stress tests like warming cooling cycle test, centrifugation test and freeze defrost pressure tests. It was found that formulation NE3 was more stable in centrifugation test and are submitted for further characterization and evaluation. The transmittance study of prepared nanoemulsion composition study was done for optimization of clarity of emulsion. The clarity of nanoemulsions was checked by transparency, measured in terms of transmittance (%T). Formulation FN2, FL2 and FT2 has 99.36%, 99.45% and 99.21% respectively transmittance values greater than 99%. These results indicate the high clarity of nanoemulsion. Due to higher particle size, oil globules may reduce the transparency of nanoemulsion and thereby values of % Transmittance.

Table 9: Evaluation of base gel formulation

Parameters	Formulations							
	C9341	C9342	C9343	C9344	C9701	C9702	C9703	C9704
Feel of application Skin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Spreadability (g.cm/sec)	13.2	12.5	10.7	10.1	8.9	7.4	6.9	5.7
Consistency	Poor	Good	Good	Excellent uniform	Poor	Poor	Fairly good	Good
pH	6.22	6.43	7.01	7.54	6.88	7.88	7.98	8.01
Viscosity (cps)	0.88	0.91	0.95	1.06	0.99	0.94	0.88	0.76
Extrudibility	Good	Good	Excellent	Excellent	Poor	Poor	Good	Good

In-vitro Anti-fungal activity study

The result of in-vitro anti-fungal study was shown in **Table 10** and **Figure 10**. The result indicated that the prepared formulation TNEG1 was best over all the formulations because of its more ability of zone of inhibition on both the microorganism naming i.e. Tinea Pedis & Tinea Cruris.

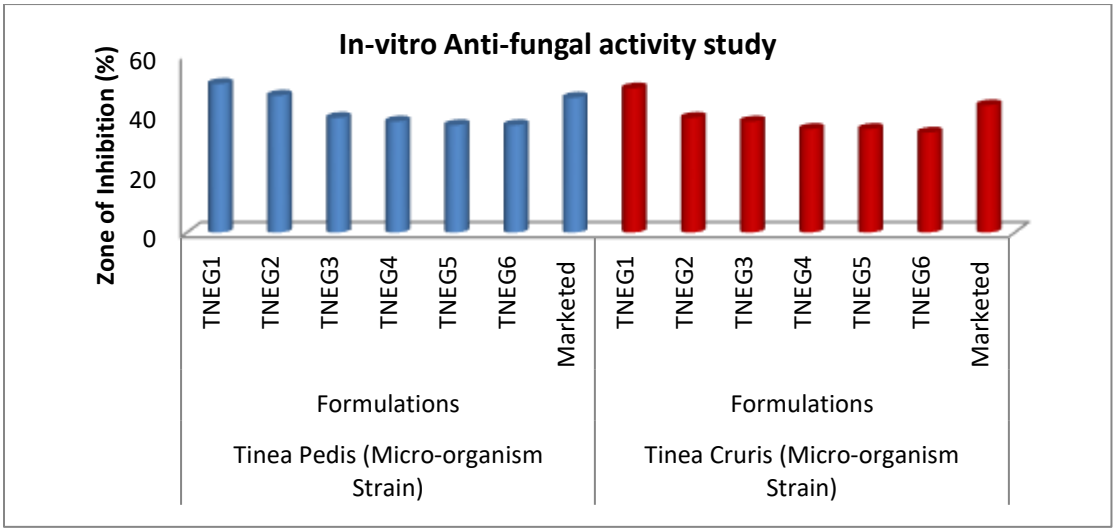


Figure 10: In-vitro Anti-fungal activity study

Table 10: In-vitro Anti-fungal activity study

Micro-organism	Formulation	L1 (cm)	L2 (cm)	Zone of inhibition (%)
Tinea Pedis	TNEG1	8	4.01	50.2±1.12
	TNEG2	8	3.7	46.25±1.04
	TNEG3	8	3.1	38.75±1.30
	TNEG4	8	3	37.5±1.09
	TNEG5	8	2.9	36.25±1.23
	TNEG6	8	2.9	36.25±1.08
	Marketed	8	3.9	48.75±1.60
Tinea Cruris	TNEG1	8	3.9	48.7±1.21
	TNEG2	8	3.1	38.75±1.20
	TNEG3	8	3	37.5±1.06
	TNEG4	8	2.8	35±1.23
	TNEG5	8	2.8	35±1.06
	TNEG6	8	2.7	33.75±1.16
	Marketed	8	4	43.0±1.25

In-vivo evaluation: The albino Wistar rats (Male) of 150 ± 20 g weiged size were used for topical fungal cure studies. We collect 24 animals from house and divided into four groups containing 6 animals each. Group I animals were take

care of marketed cream with tolnaftate drug as positive control, whereas Group II act with finalized optimized formulation nano-emul-gel TNEG1 respectively. The animals were maintained at a temperature of 21 ^{0}C and were allowed access to feed and water. T. Pedis was an infectious agent and used for the study of susceptibility to fungal infection than T. cerius. The micro-organism was obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. The obtained fungal species were maintained on Sabouraud Dextrose Agar (SDA) at 4 ^{0}C, and sub cultured once a month.

The dorsal surface of each animal having an area of 5 cm^2 was depilated and applied 7 day old cultured infectious inoculum of **T. Pedis** on it and left for 3 days. The presence of scales and redness as initial symptom oneach animals on the 5th day was observed. Now the treatment was started on the 5th day post inoculation until complete recovery from the infection was achieved. The therapeutic efficacy of the nano-emul-gels was evaluated on daily basis by macroscopic examination of lesion. The result was shown in **Table 11** and **Figure 11**.

Table 11: in-vivo animal pharmacokinetic study

Time (h)	Marketed Cream	TNEG1
2	1.23	2.34
4	1.67	3.23
6	3.45	5.34
8	5.34	6.56
10	7.23	11.23
12	9.76	15.67
14	14.67	9.76
16	21.56	7.67
18	6.34	5.34
20	0.91	2.01
22	0.87	0.652
24	0.087	0.023

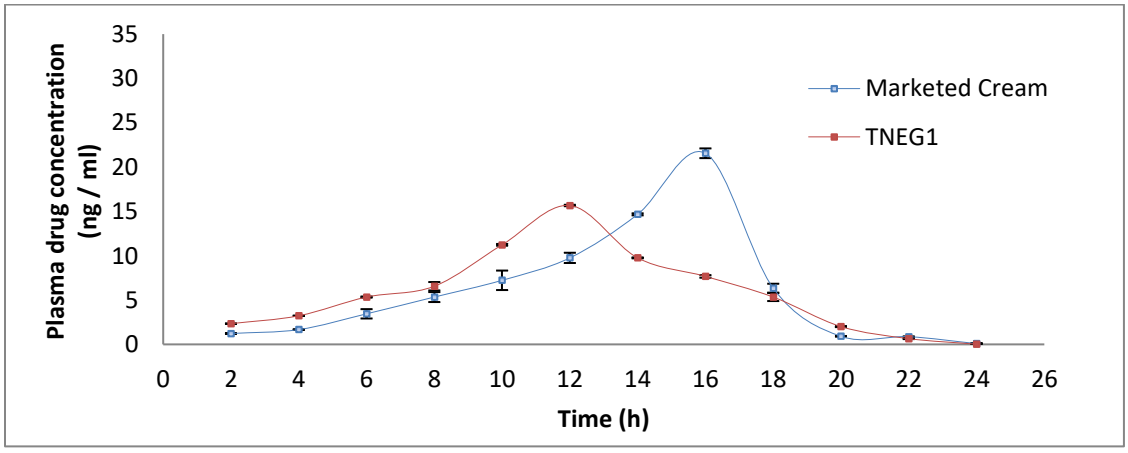


Figure 11: In-vivo animal pharmacokinetic study

The pharmacokinetic study of prepard formulation TNEG1 and marketed preparation were performed in guinea pig. The marketed preparation and prepared formulation (TNEG1) was applied on infectious area. It gets about 5th h for drug may appear in plasma and resultant shown quick absorption of the drug. The peak plasma concentration (C_{max} 15.67±0.19 ng/ml) at 5th h (T_{max}) of prepared formulation (**Table 11**), which will equivalent to use marketed formulation (**Figure 11**).

Stability studies

The nano-emulgel (TNEG1) are the semi-solid topical drug delivery system were prepared with various polymeric blend and oils at temperature 2±2°C, 25°C & 60% RH and 40°C & 75% RH, 50°C & 75% RH, 60°C & 75% RH for a period of 180 days. The stability of a formulation is known as the power of the materials to stay on inside definite restrictions over a fixed phase of time and known as shelf life of the product. The data identified that the TNEG1 stored at temperature 25°C & 60% RH, informed, it was less than 5 % degradation at the end of six months. It may indicate that the formulations could provide a minimum shelf life of 2 years.

Table 12: Stability studies

S. No.	Temperature (°C)	Room humidity (RH)	Residual drug content (%)				
			Time in days				
			0	30	60	90	180
1	2°C	-	99.92±0.08	99.91±0.03	99.87±0.11	99.82±0.06	99.78±0.03
2	25°C	60% RH	99.92±0.08	99.81±0.02	98.87±0.05	98.11±0.08	97.2±0.06
3	40°C	75% RH	99.92±0.08	99.11±0.05	98.02±0.06	97.36±0.07	97.02±0.07
4	50°C	75% RH	99.92±0.08	99.10±0.03	98.01±0.02	97.01±0.12	96.98±0.14
5	60°C	75% RH	99.92±0.08	99.01±0.08	97.98±0.13	96.87±0.12	96.43±0.07

Conclusion

The present investigation is to develop nanoemulgel of tolnaftate as anti-fungal drug with better-permeable, controlled and localized delivery via topical route. Tolnaftate is used to treat skin infections such as athlete's foot, jock itch, and ringworm. The absorption maxima of drug were determined by scanning drug solution in double beam ultraviolet spectrophotometer between 200 to 400 nm wavelengths. The various Preformulation parameters were studied**.** The Tolnaftate (TFT) nanoemulsion was prepared by the high speed homogenization method and various parameters were evaluated **i.e.** Thermodynamic Stability (Heating cooling cycle, Centrifugation, Freeze thaw cycle, Robustness to Dilution), Transmittance Measurement etc. Nanoemulsions were found thermodynamically stable systems and were formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. The prepared formulation as NE3 formulations were stable in centrifugation test and are submitted for next characterization and evaluation. The gel base containing Carbopol 970 (C01-C04) showed phase separation and poor in consistency as indicated by spreadability and extrudability values, so these gel base formulations were rejected for next level. The

result of in-vitro anti-fungal study indicated that the prepared formulation TNEG1 was best over all the formulations because of its more ability of zone of inhibition on both the microorganism naming i.e. Tinea Pedis & Tinea Cruris. The pharmacokinetic study of prepard formulation TNEG1 and marketed preparation were performed in guinea pig. The marketed preparation and prepared formulation (TNEG1) was applied on infectious area. It gets about 5^{th} h for drug may appear in plasma and resultant shown quick absorption of the drug. The peak plasma concentration (C_{max} 15.67±0.19 ng/ml) at 5^{th} h (T_{max}) of prepared formulation, which will equivalent to use marketed formulation. The nano-emulgel (TNEG1) are the semi-solid topical drug delivery system were prepared with various polymeric blend and oils at temperature 2±2°C, 25°C & 60% RH and 40°C & 75% RH, 50°C & 75% RH, 60°C & 75% RH for a period of 180 days. The stability of a formulation is known as the power of the materials to stay on inside definite restrictions over a fixed phase of time and known as shelf life of the product. The data identified that the TNEG1 stored at temperature 25°C & 60% RH, informed, it was less than 5 % degradation at the end of six months. It may indicate that the formulations could provide a minimum shelf life of 2 years.

References

1. Chen H; Khemtong C; Yang X; Chang X; Gao J; 2011; Nanonization strategies for poorly water soluble drugs; Drug Discov Today; 16; 7–8; 354–360.
2. Choudhury H; Gorai B; Pandey M; Chatterjee LA; Sengupta P; Das A; Molugulu N; Kesharwani P; 2017; Recent update on nanoemulgel as topical drug delivery system; Journal of Pharmaceutical Sciences; S0022-3549(17; 30232-0; .
3. Fujita S; 1997; Animal models of dermatomycoses in recent years; Jap J Med Mycol; 38; 33–37
4. Gungor S; Erdal MS; Aksu B; 2013; New Formulation Strategies in Topical Antifungal Therapy; Journal of Cosmetics; Dermatological Sciences and Applications; 3; 56-65.
5. Jain A K; Jain C P; Tanwar Y S; Naruka P S. Formulation; characterization and in vitro evaluation of floating microspheres of famotidine as a gastro retentive dosage form. Asian Journal of Pharmaceutics. 2009; 3; 3; : 222-6.
6. Karri VVS; Raman SK; Kuppusamy G; Mulukutla S; Ramaswamy S; Malayandi R; 2015; Terbinafine hydrochloride loaded nanoemulsion based gel for topical application; Journal of Pharmaceutical Investigation; 45; 79-89.
7. Karri VVSR Raman SK; Kuppusamy G; Sanapalli BKR; Wadhwani A; Patel V; Malayandi; 2018; In vitro Antifungal Activity of a Novel Allylamine Antifungal; Nanoemulsion Gel; Journal of Nanosciences: Current Research; 3; 1; 1 – 5.
8. Kotta S; Khan AW; Ansari SH; Sharma RK; Ali J; 2014; Anti HIV nanoemulsion formulation: Optimization and in vitro-in vivo evaluation; Int. J. Pharm.; 462; 129–134.
9. Lachman/Lieberman's; 2004; The Theory and Practice of Industrial Pharmacy; CBS Publishers & Distributors Pvt Ltd; Fourth Edition; 680-686.
10. More A and Ambekar AW; 2016; Development and Characterization of Nanoemulsion Gel for Topical Drug Delivery of Nabumetone; International Journal of Pharmacy and Pharmaceutical Research; 7; 3; 126-157.
11. Panwar AS; Upadhyay N; Bairagi M; Gujar S; Darwhekar GN; Jain DK; 2011; Emulgel - a review; Asian J. Pharm. Life Sci; 1; 2231–4423.
12. Raymond C; Paul J; Sian C. Handbook of Pharmaceutical Excipients. 5th edition. PhP APhA Pharmaceutical Press. 2006. P 246-278.
13. Srpska M; Sad N; Serbia B; Johnson SE; Academy MM; 2007; Experimentally induced dermatomycoses at rats and treatment with Lavandula angustifolia essential oil; Proc Nat Sci; 113; 249–254.
14. Stephen T; Skillen DM; Bickley L; 2010; Bates guide to health assessment for nurses; Philadelphia: Wolters Kluwer.
15. Van Cutsem J; 1989; Animal models for dermatomycotic infections; Curr Top Med Mycol; 3; 1–35.
16. Vats S; Saxena C; Easwari TS; Shukla VK; 2014; Emulsion Based Gel Technique: Novel Approach for Enhancing Topical Drug Delivery of Hydrophobic Drugs; International Journal for Pharmaceutical Research Scholars; 3; 2; 649-660.