WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 14, 965-974. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF NANOEMULSION COMPRISING OF BERBERIS ARISTATA AND CROTON TIGLIUM AS EFFECTIVE TREATMENT FOR TINEA CAPITIS FUNGAL INFECTION

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Article Received on
27 Sept 2021,

Revised on 17 October 2021,
Accepted on 07 Nov 2021

DOI: 10.20959/wjpr202114-22274

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ABSTRACT

Tinea capitis is a superficial fungal hair follicle infection in the scalp. The disease usually caused by dermatophytes in the genera Trichophyton and Microsporum that attack the hair shaft. The present study is based on formulation of nanoemulsion containing extracts of *Berberis aristata* and *Croton tiglium* as effective treatment to treat the *Tinea captis* fungal infection. Presently no such effective treatment is available in the market to treat the mentioned infectious disease. The nanoemulsion additionally offers several benefits like better penetration and bioavailability for transdermal application.

KEYWORDS: *Tinea capitis*, Nanoemulsion, *Berberis aristata* and *Croton tiglium*.

INTRODUCTION

Tinea capitis Tinea capitis is a superficial fungal hair follicle infection in the scalp. Some dermatophytosis as no susceptibility to age like Tinea capitis which almost always involves the pediatric age group. In developing countries the incidence is high due to factors such as overcrowding poverty, poor sanitation and illiteracy. Tinea capitis is a disease that results from superficial fungal infection of the skin of the scalp, with a tendency to attack hair shafts and follicles, eyebrows and eyelashes.[1,2,8,9,10,11,12,14] The clinical appearance is typically single or multiple hair loss spots, sometimes with a pattern of ' black dot ' (often with missing

hair), which can be followed by swelling, scaling, pustules, and scratching. Tinea capitis, uncommon in adults, is seen mostly in prepubertal babies, boys more often than girls. Capitis tinea is contagious infection.[1,2,3,8,10,11,12,14] The infection is distributed through close contact with an infected person, or through sharing with someone infected with combs, hairbrushes, caps, shoes, sheets, beds and other things. Ringworm can also be catched from infected animals such as dogs, cats, horses or farm animals.[3,4,8,9,10,14] The fungus can remain in the atmosphere for long periods of time, and therefore infection can occur several months later. Nanoemulsion is one of the supreme and important dosing types with of nanotechnology in pharmaceutical formulations. It is the novel drug delivery mechanism that allows for the safe or controlled release of medicine, biological active ingredient.[3,4,13,15] Nanoemulsion is a mixture of oil, surfactant and aqueous, isotropically transparent and thermo-dynamically or kinetically stable, Very small sized Nanoemulsion droplets helped enhance drug absorption and targeting.[4,5,13,15] Nanoemulsions, also known as submicron emulsions, ultrafine emulsions and miniemulsions, are submicron-sized colloidal particulate structures considered as thermodynamically and kinetically stable isotropic dispersions, consisting of two immiscible liquids such as water and oil, stabilized in one step by an interfacial film consisting of an effective surfactant and co-surfactant.[4,6,13,15] Because to its small scale, it has the ability to cross the biological membrane and there, by increasing the therapeutic efficacy of a drug molecule.[4,7,13,15] The present examination was gone for building up a Nanoemulsion of *Berberis aristata* and *Croton tiglium* for treatment of Skin infections like *Tinea capitis* and to screen the antifungal activity of the prepared formulation.

MATERIALS AND METHODS

Preformulation is the first initial phase in basis improvement of any pharmaceutical and ayurvedic dose structure in another formulation since preformulation study centers around those novel approaches that can partner impact on medication execution and advancement of a various dosage forms. In the Preformulation study concluded that, the material suitable in theAyurvedic and pharmaceutical formulation.

Organoleptic properties- This includes testing of properties like color, taste, odour, etc.

Melting point determination- Melting point assurance of Berberis aristata and croton tiglium had decided by open slimcylinder strategy utilizing Thieles tube device.

Spectroscopic analysis- Stock arrangement of 100µg/ml was set up by including 10mg of

unadulterated Berberis aristata and croton tiglium in 10mL of dissolvable Methanol. At that point, 1mL of stock arrangement had taken and appropriately weakened with arrangement of methanol to make 10 µg/ml of Fluconazole arrangement. The arrangement was then sifted and its UV range wasrecorded in the wavelength go 200 - 400 nm.

Preparation of calibration curve for berberis aristata- Stock solution of 100µg/ml was prepared in solvent methanol and further diluted with solution of methanol. As solvent to get solutions with concentration range 5-25 µg/mL. The solutions were then filtered and analyzed spectrophotometrically at 260 nm.

Fourier transform infrared spectrometry (FTIR)- FTIR analysis can be carried out for the assessment of drug excipient interaction, polymerization, cross linking as well as drug loading in the formulation. It is also used for identifying the functional groups with their means of attachment and the fingerprint of the molecule. At low temperature a molecule exists in ground state and on absorbing the radiant energy, they get excited to higher energy states. IR spectroscopy is based on determining this energy difference (ΔE) between the excited and ground states of the molecule. For performing FTIR, sample can be prepared by employing suitable method such as potassium bromide pellet method, Nujol mulls and then sample is scanned in FTIR at moderate scanning speed between 4000- 400 cm-1(59) Pattern of drug due topresence of ingredients was investigated to identify any chemical interaction.

Interpretation of IR spectrum of *Berberis Aristata* and *Croton tiglium*- The infrared spectrum of pure Berberis aristata powder and croton tigium liquid was recorded and its spectral analysis was done.

Compatibility studies between Drug and Excipients- Incompatibility is the consequence combination of two extract of at least two or more than two substances and is recognized by physical, herbal helpful characteristics. It might influence thewellbeing, viability and presence of the measurement structure. It is subsequently of prime significance for detailing researcher to decide conceivable incongruence between dynamic fixings and excipients use to make last measurement structure. In this investigation, we inspected infrared examination to distinguish any connection (compound or physical) or development herbal drugs among medication and various surfactanctants

Formulation of nano emulsion- Formulation of nanoemulsion was prepared by the high

pressure homogenization. Nanoemulsion formulation has been optimized in terms of surfactant size, concentration andratio, oil water ratio, and the high energy input technique. In Nano emulsion Preparation balance thoroughly, organic phase and aqueous phase remained under magnetic stirring at specific temperature and revolutions per minute (rpm) for 20 minutes. Applied drop by dropusing a syringe to the uniform mixer required quantity of water and tween 20 mixture provided the mixer was held in the magnetic stirrer, resulting in yellowish colored emulsion formation. Composition of *Berberis aristata* and *Croton tiglium* loaded Nanoemulsion as per Central Composite Design is summarized in Table no: 01.

Procedure of preparation of nano emulsion-

- Aqueous phase -Herbal extract, tween80 dissolved in water. Stirring of phase was done until the formation of aqueous phase.
- Oil phase- Oil dissolved in polyethylene glycol Both Mixed with each other with continuous stirring until the formation of oil phase.
- Both aqueous and oil phase mixed with each other at room temperature with continuous onmagnetic stirrer at 30 min. Finally added methyl paraben with continuous stirring was done until the Nanoemulsion formulation.

Evaluation of nanoemulsion

Appearance- The prepared emulsions were investigated outwardly for clearness with yellowish shading and nearness and stability of any molecule. The test is significant with respect to persistent consistence.

Ph- pH of nanoemulsion was resolved utilizing pH meter The pH meter is used for measuring the pH of a nanoemulsion and microosmometer is used for determining the osmolarity of emulsion, which is based upon freezing point method. For performing this, 100 µl of nanoemulsion is transferred in microtube and measurements are taken.

Viscosity determination- The consistency of the emulsion arranged was resolved utilizing Brookfield viscometer model (LVDV-II+), the gel test was filled in the example holder and the specific axle drenched into the example, the axle is joined to the viscometer and after that it is permitted to pivot at a specific speed then thickness of the plan was estimated following 2 minutes.

Drug content- 0.5 ml of the prepared Nanoemulsion was diluted with 10 ml of ethyl acetate and filtered with a 0.45 µm filter. Total drug content was determined by UV spectrophotometry at 260 nm.

Entrapment efficiency- Total amount of drug present in Nanoemulsion was determined by adding 10ml in 10ml of methanol. Drug was extracted in methanolic phase solution, filtered the solution and absorbance was determined by UV spectrophotmetrically at 260 nm.

Particle size - The molecule size of the readied Nanoemulsion was estimated by utilizing a Nano Partica Nano Particle Analyzer Horiba Scientific SZ - 100. It was seen that the normal molecule size was observed to be 85.3 nm.

Zeta potential- Zeta potential for Nanoemulsion was resolved utilizing Nano Partica Nano Particle Analyzer Horiba Scientific SZ - 100. Test was put in clear expendable zeta cells and results were recorded. Prior to putting the new example, cuvettes were washed with the methanol and flushed utilizing the example to be estimated before examination.

Refractive index- Refractive index of nanoemulsion measured by Abbes Refractometer.

Polydispersity- This shows the uniformity of the size of the droplet in the nanoemulsion. The higher the amount of polydispersity, the lower the uni-formity of Nano-emulsion droplet size. It can bedefined as the ratio of the standard deviation to the average droplet size.

***In vitro* drug release study-** Topical emulsions definitions were required to discharge the medication immediately when they are connected to the skin for a speedy alleviation. To test the example of arrival of medication from definitions in vitro dissemination studies were completed. The mechanical assembly comprise of a barrel shaped glass tube (with 22mm inward distance across and 76 mm stature) which was opened at both the ends.1 ml of emulsion plan was spread consistently on the outside of Cellophane layer (recently absorbed water for medium-term) and was fixed to the one finish of cylinder to such an extent that the readiness possesses internal boundary of the cylinder. The entire get together was fixed so that the lower end of cylinder containing emulsion was simply contacted (1-2 mm profound) the outside of dissemination medium i.e., 25ml pH 7.4 phosphate cradle contained in 25 ml beneficiary which was place underneath the benefactor compartment and kept up at 37±2ºC. The cellophane film goes about as a boundary between the emulsion stage and pH 7.4 phosphate cradles (sink stage). An amount of 1ml examples were pulls back from receptor liquid at the time interim of[1,2,3,4,5,6] hrs. The discharged medication was evaluated by utilizing Shimadzu UV–unmistakable spectrophotometer at 260 nm and 1 ml phosphate cushion pH 7.4 wassupplanted each time. Dissolution Medium was Phosphate Buffer (pH 7.4) at 50 RPM.

Accelerated stability studies of nanoemulsions - Preliminary stability of the nano-emulsion preparation was checked in 24-hour centrifugal and thermal stress analyzes. Stability was measured by evaluation of the macroscopic emulsion and study of the size of the droplet. The purpose of these tests was to choose a safe, low-surfactant formulation with a nano-emulsion-

sized droplet and safe physicochemical properties. The selected nano-emulsion was prepared in triplicate and the samples were deposited at 25 ± 2 °C, 40 ± 2 ° C and 5 ± 2 ° C. Tests were conducted 24 hours, 7, 15, 30, 60 and 90 days after planning.Measurements of the study were droplet size, pH value and electrical conductivity.

Antifungal activity- Antifungal activity against fungus trycophyton rubrum fungus produced in the *tiniea capitis* diseaseon the production or formulation of nanoemulsion was produced from herbal medicinal plants like*berberis aristata* and *croton tiglium*. In the work formulation was produced and activity checked on the nanoemulsion formulations andother dosage forms.

RESULTS AND DISCUSSION

Organoleptic properties: Organoleptic properties of Berberis aristata were color Pale Yellow, Odour Slightly phenolic and taste slightly bitter. Organoleptic properties of Croton tiglium color light brown, odour and taste both were slightly unpleasant.

Melting point: Temperature was noted at which strong medication changes of *Berberis aristata* was observed to be 120^0 C. The melting point of *Croton tiglium* was to be 63^0c

Preparation of standard curve of *Berberis aristata*- The standard arrangement of *Berberis aristata* of focus 100µg/ml demonstrated most extreme absorbance at the wavelength of 260 nm. λ max of *Berberis aristata* was observed to be 260 nm.

Evaluation of nanoemulsion

Appearance test- All the batches F1 to F9 were clear in appearance.

pH test: The readied *Berberis aristata* nanoemulsion definitions were clear gooey rich arrangement with a smooth and homogeneous appearance. The pH estimations of all readied detailing ran from 5.5 to 5.7, which are viewed as adequate to keep away from thedanger of bothering upon application to the skin since grown-up skin pH is 5.5.The pH of different plans appears as follows F1-5.5 ±0.01, F2-5.79 ±0.01, F3- 5.65±0.02, F4- 5.28 ±0.03, F5-5.45 ±0.02, F6-5.45 ±0.02, F7-5.75 ±0.02, F8-5.56 ±0.01 and F9-5.65±0.01**.**

Spreadability test: The Spreadability of different definitions was found between 8.69-12.42gm/cm2. It is appeared in as F1-10.98±0.2, F2-10.9±0.4, F3-12.5±0.3, F4- 11.54±0.2, F5- 9.28±0.2, F6- 10.29±0.3, F7-9.54±0.2, F8- 8.69±0.4, F9-12.42±0.3.

Extrudability test- All the readied definitions demonstrated great Extrudability and it was found between 3.7-4.2gm/cm2. The outcomes are appears F1-3.8, F2-3.7, F3-4.2, F4-3.9, F5-4.0, F6-3.6, F7- 4.1, F8-4.0 and F9-3.8.

Viscosity determination: The estimation of consistency of the readied Nanoemulsion was

completed with Brookfield viscometer (Brookfield DV-E viscometer). The definition was pivotedat 10 (min.) and 100 (max.) revolution every moment with axle 64. At each speed, the comparing dial perusing was noted, and the consistency of different plans was observedto be 8820-10022 cps as appears F1-9320, F2-9692, F3-9198, F4-9081, F5- 8820, F6-10022, F7-8950, F8-9856 and F9-9485.

Refractive index- The estimation of the refractive index of nanoemulsion measured by Abbe's refractometer. It is usedfor the determine index of the refraction of the formulation. The refractive index of all batches are given below F1-1.6050, F2- 1.6330, F3- 1.6320, F4-1.6280, F5-1.6059, F6- 1.6520, F7-1.6240, F8-1.6052 and F9-1.6035.

Polydispersity- It is depends on the particle size of the nanoemulsion formulation. PDI is used to calculate the width ofthe molecular weight distribution of the polymer.it is defines as the average weight of molecular weight F1-0.21, F2-0.25, F3-1.25, F4-0.15, F5-1.52, F6-2.58, F7-1.24, F8-0.27 and F9-1.45.

Drug content- Drug substance was determined utilizing the condition, which was gotten by direct relapse investigation of adjustment bend. The medication substance of different plans was seen between 95.6-98.2% F1-98.2±0.3, F2-96.2±0.4, F3-97.8±0.4, F4-95.6±0.4, F5-96.2±0.2, F6-98.3±0.2, F7-96.5±0.5, F8-98.2±0.5 and F9-95.6±0.2.

Entrapment efficiency- Total measure of medication present in Nanoemulsion was controlled by including 10ml of gel in 10ml of methanol. Medication was extricated in methanolstage arrangement, separated the arrangement and absorbance was dictated by UV spectrophotometrically at 260 nm. The Entrapment productivity of different plans is appeared in Table no 8.13 and was found between 43.5-69.2%. Entrapment Efficiency of Various formulations F1-43.5; F2-62.5; F3-68.2; F4-56.25; F5-65.2; F6-68.2; F7-69.2; F8-56.2 and F9-56.3.

Particle size- The mean molecule size expanded with expanding polymer focus. Expanding polymer fixation created a huge increment in the thickness, bringing about combination of semisolid particles and delivering a generally speaking expanded in size of Nanoemulsion.The molecule size of the improved definition (Batch F1) was observed to be 220 nm. The strength investigation of the readied Nanoemulsion of berberis aristata and croton tiglium was assessed by estimating the zeta capability of the Nanoemulsion. It was observed to be - 0.1 mV. Cluster F1 demonstrated high zetapotential worth which showed better dependability of item, as the particles keep on repulsing one another and to a great extent stay in a non-accumulated state.

***In vitro* drug release-** The result of *in vitro* percentage amount of drug released at different time intervals is plotted against time to get the discharge profiles, as appeared in Figure no 1. The % medication discharge relies upon increment of centralization of the polymer. The medication discharge was recorded between 81-98%.

Stability study of the optimized formulation (F1)- During the solidness examines no huge change was seen in the physical appearance,thickness, tranquilize content, spreadability, ensnarement productivity, in the Nanoemulsion definition following 3 months soundness investigation of detailing F1. The strength information for advanced detailing F1 is given in Table no. 2. This demonstrates the all plans were not especially influenced by the adjustments in the temperature and moistness. In this manner, no proof of corruption of medication was watched.

Antifungal activity of nanoemulsion- Powder extract of the dried leaves of *Berberis aristata* and *Croton tiglium* showed antifungal activity in dose dependent manner giving shortest time of growth and its death or destroy fungus. The effect of ethanol as solvent was also determined by taking pure solvent in the study. The activity showed during the experiment may be the result of the various formulations like Nanoemulsions creams herbal Nano cream and branded creams. The study concluded that the effect of the nanoemulsion is more as compare to the other formulations. Zone of inhibition of the of *Berberis aristata* and *Croton tiglium* nanoemulsion formulation is 2.5cm.

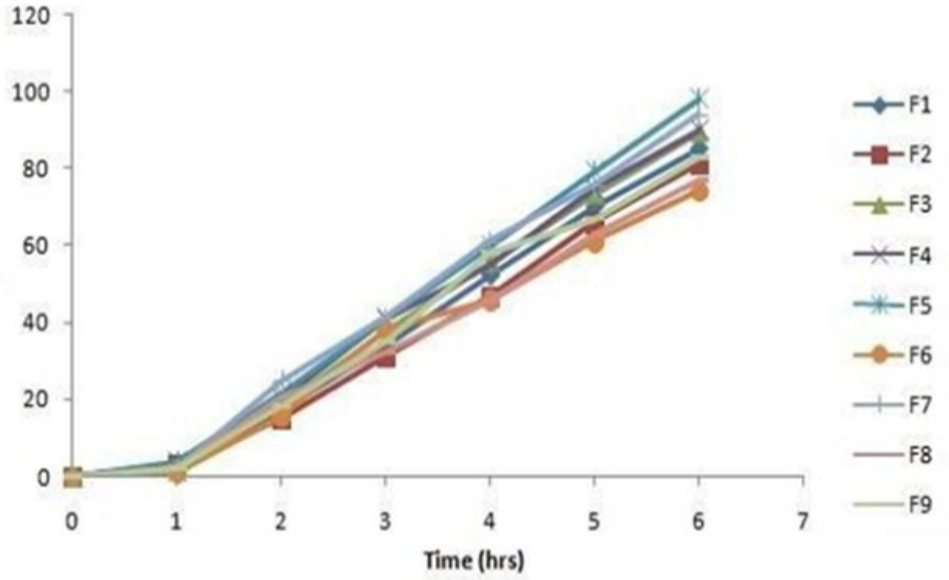

Figure no. 01: In vitro percent drug releases for all batches (F1-F9) represented as Mean ± SD (n=3).

Table no. 01: Composition of *Berberis Aristata* and *Croton tiglium* loaded Nanoemulsion as per Central Composite Design.

Batchno.	*Berberis aristata* (gm)	*Croton tiglium* (ml)	Orange oil (ml)	Tween 80 (ml)	PEG400 (ml)	Water(ml)
F1	10	6	6.6	18.05	6.05	28.08
F2	10	6.5	5	18.02	6.05	29.1
F3	10	6.1	6.5	18.05	6.05	28.08
F4	10	6	6.6	18.05	6.05	28.08
F5	10	6.2	6.2	18.05	6.05	28.08
F6	10	6	6.6	18.08	6.05	28.08
F7	10	6	6.6	18.08	6.05	28.07
F8	10	5.9	6.2	18.08	6.05	28.09
F9	10	6	6.3	18.08	6.05	28.08

Table no. 02: Stability studies of optimized formulation (batch F1) represented as Mean ± SD (n=3).

Sr. No.	Parameters	At temp. 40^0C ± 2^0C, RH 75% ± 5%			
		Zero month	One month	Two months	Three months
1.	Physical appearance	Clear	No change	No change	No change
2.	Viscosity	9387	9387	9285	9387
3.	pH	5.5±0.2	5.6±0.1	5.5±0.1	5.5±0.2
4.	Refractiveindex	1.6050	1.6049	1.6050	1.6050
5.	Drug content	98.2±0.2	98.3±0.3	98.2±0.2	98.3±0.1
6.	% *In vitro* drug release	85±0.1	86±0.2	85±0.3	85±0.2
7.	Entrapment Efficiency	43.5	43	43.5	43
8	Spread ability	10.98±0.1	10.90±0.2	11.20±0.2	10.99±0.3

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