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A NEW PERSPECTIVE FOR THE TREATMENT OF DANDRUFF & ASSOCIATED ALOPECIA WITH EMULSION BASED GEL CONTAINING KETOCONAZOLE AND MINOXIDIL

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ABSTRACT: Many antifungal drugs are used topically in order to treat fungal diseases. Among them Ketoconazole is used widely among these topical antifungal drugs for eradication of dandruff. Scalp with dandruff causes more hair fall than normal scalp and hair fall is treated by topical Minoxidil formulation. Aim of present work is to prepare a topical formulation containing both ketoconazole and minoxidil in order to treat dandruff and associated diffused alopecia simultaneously. Both the drugs are incorporated in emulgel at a particular step in order to prepare formulation. Emulgel i.e. emulsion in gel have been emerged out as one of the most beneficial topical drug delivery system as it contain both hydrophilic and lipophilic drugs with dual controlled release system i.e. emulsion and gel. Incorporation of emulsion in gel also increases the stability of emulsion. Emulgel was prepared by using carbopol 934 as gelling agent. The influence of concentration of gelling agent and emulsifying agent on the drug release from the prepared emulgel was investigated using a 2×2^2 factorial design. The prepared emulgel were evaluated for their physical appearance, viscosity, pH, spreadability, drug content and drug release. All the prepared emulgel showed acceptable physical properties concerning color, homogeneity, consistency, spreadability and pH value. The highest release was observed with Formulation F8 (92.65±0.08 % for Ketoconazole and 97.63±0.27 % for Minoxidil) and lowest with Formulation F1 (31.39±0.28 % for Ketoconazole and 40.66±0.19 % for Minoxidil). Since we need lowest Ketoconazole release in circulation and highest deposition in skin for dandruff eradication, hence Formulation F1 was further evaluated for drug deposition in rat skin via indirect method and 41.62 % of Ketoconazole and 15.83 % of minoxidil was observed. Thus this formulation can be used for simultaneous treatment of dandruff and associated diffused alopecia for better patient compliance.

INTRODUCTION: Dandruff is a common scalp disorder which affects both male and female population. It is a common complaint among half of the population of the world.

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It can be diagnosed by presence of flakes on scalp and among hairs, may or may not be associated with itching. It occurs after puberty, irrespective of ethnicity and gender [1]. Severity of dandruff may vary with seasons in subjects whose scalps are prone to dandruff and it gets worst in winter [2].

There are many etiopathogenic pathways with complex mechanisms, which cause dandruff. Dandruff is a fungal infection; particularly the *Malassezia spp*. plays a key etiologic role in developing dandruff. They target the corneocytes

of scalp. Susceptibility of corneocytes for these fungi varies on the same scalp. Substantial evidence indicates that keratinocytes play an active role in the generation and expression of immune-pathological reactions. Malassezia yeast stimulates the colonization of corneocytes which results in release of pro-inflammatory mediators and growth of subclinical micro inflammation present in dandruff. Human scalp normally harbours many micro-organisms thus it is a common habitat for Malassezia yeast but their count increases by many folds during dandruff. Their density reaches 103 to 105 organisms per mm^2 [1-5]. Factors supporting this hypothesis are: (i) antifungal treatment is effective in treating dandruff (e.g. zinc pyrithione, selenium sulphide and ketoconazole), and (ii) improvement in dandruff is accompanied by a reduction in Malassezia levels on the scalp. It has been reported that the patients with dandruff have higher proportion of Malassezia cells on their scalp than on normal controls [1, 4, 8].

Chronic dandruff is able to precipitate telogen effluvium (thinning and shedding of hair due to early entry of hair in telogen phase) and aggravate androgenetic alopecia [2]. Androgenetic alopecia, or patterned alopecia, is a common form of hair loss in both men and women which is characterized by a progressive loss of hair thinning of hairs, shortening of length, and loss of pigmentation. The genetic inheritance of androgenetic alopecia is well known, although the causative genes have yet to be elucidated. Androgenetic alopecia is often precipitated and exacerbated by conditions that can induce telogen effluvium [6, 7] On a two-day survey, it has been observed that about 100-300 numbers of hairs were shed in dandruff sufferers instead of 50-100 in normal subjects [2].

In some cases of dandruff, hair shedding may be a result of alterations in the teloptosis process (exogen phase) and hair eclipse phenomenon. Interestingly, some of the antidandruff compounds, especially ketoconazole, may limit the progression of androgenic alopecia [9-11].

The anti-fungal agent ketoconazole is used topically for the treatment of dandruff [1, 12]. There is some evidence, both in humans [13] and in rodents [12], that this agent may stimulate hair growth. The mechanism is unknown, but may involve inhibition of inflammation, or anti-androgenic properties of the agent. To date the evidence is based on small sample sizes and does not include clinical trials. Thus, further research is required to determine its efficacy [14].

The U.S. Food and Drug Administration (FDA) have labeled topically administered minoxidil for the treatment of androgenetic alopecia. A dropper is used to apply minoxidil solution directly onto dry scalp twice daily [6, 14, 15, 16].

Now it's the need to develop a formulation containing both drugs ketoconazole and minoxidil for treatment of dandruff and associated androgenetic alopecia simultaneously. Ketoconazole can supports minoxidil therapy As ketoconazole is effective for the treatment of dermatitis and dandruff, and its action on scalp microflora may benefit those with androgenetic alopecia associated follicular inflammation, as it may stimulate hair growth [13]. However, ketoconazole is also an anti-androgen and has been suggested to improve hair growth in androgenetic alopecia through androgen dependent pathways [17].

MATERIALS AND METHODS:

Materials: Ketoconazole was received as a gift sample from Gufic Bioscience Ltd Mumbai and Minoxidil was received as gift sample from Marico Ltd. Chennai. Carbopol 934; Liquid paraffin; Tween 20; Span 20; Propylene glycol; Methyl paraben; Propyl paraben were purchased from Loba Chemie , Mumbai. Ethanol was purchased from C. Y. Company China. Double distilled water was used for all experiments. All chemicals were pharmaceutical grade and used without further modification.

Analytical method: Ketoconazole and minoxidil in the samples was estimated by a UV spectrophotometric method. Drugs in the samples were collected from in vitro and ex-vivo release studies and analysed by a UV spectrophotometer. Calibration curve was obtained by weighing an accurate amount of Ketoconazole and solubilize in the methanol. Primary standards in the concentration range of 5 to 30 µg/ml was obtained on suitable dilutions and the calibration curve was prepared by measuring their absorbance at predetermined λmax of 255 nm with a Simadzu-

1800 UV/visible spectrophotometer, with correlation coefficient (r^2) 0.996. The high value of correlation coefficient indicates the linearity of the calibration curve and the curve did not deviate significantly from the origin as indicated by its low value of intercept.

Similarly calibration curve of minoxidil in methanol was developed in the concentration range of 5 to 25µg/ml, by measuring their absorbance at predetermined λ_{max} of 285 nm with a Simadzu-1800 UV/visible spectrophotometer with correlation coefficient 0.998. The scanning for solution of both drug were carried out in the range of 200-400 nm against methanol as a blank for simultaneous estimation of both the drug was done by preparing their solution (10 µg/ml) in methanol (common solvent for both) and scanned in the spectrum mode over the range of 200-400 nm to obtain the overlain spectra that was used in the analysis and absorptivities ($A,1_{cm}$) for both drug at both wavelengths were determined. Concentrations in the sample were obtained by using following equations-

$$C_x = \frac{A_1\, a_{y2} - A_2 a_{y1}}{a_{x1}\, a_{y2} - a_{x2}\, a_{y1}}$$

$$C_y = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{a_{y1}\, a_{x2} - a_{y2}\, a_{x1}}$$

Simultaneous equation was developed as:

$$At\ 285\,nm \quad A_1 = a_{x1}bc_x + a_{y1}bc_y \quad \ldots\ldots\ldots\ldots\ldots(1)$$

$$At\ 255\,nm \quad A_2 = a_{x2}bc_x + a_{y2}bc_y \quad \ldots\ldots\ldots\ldots\ldots(2)$$

Where

C_x and C_y = Concentration of Minoxidil and Ketoconazole, respectively

A_1 and A_2 = Absorbance at 285 nm and 255 nm, respectively.

a_{x1} and a_{x2} = Absorption coefficient of Minoxidil at 285 nm and 255 nm, respectively.

a_{y1} and a_{y2} = Absorption coefficient of Ketoconazole at 285 nm and 255 nm, respectively.

b = 1 (for measurement in 1 cm cell)

Emulgel preparation: Emulgel was prepared by the method reported by Magdy *et al* (2004) with minor modification. Preparation of emulgel includes the preparation of emulsion phase containing Ketoconazole and an aqueous solution of gelling agent containing Minoxidil, followed by the addition of the emulsion into an aqueous solution of the gelling agent, to obtain a semisolid formulation. Gel was prepared by dissolving gelling agent in 10 ml of distilled water in which Minoxidil was dissolved. pH of the prepared emulgel was adjusted to 5.5 to 6 using Tri Ethanol Amine (TEA). The oil phase of the emulsion was prepared by dissolving the lipophilic surfactant (Span 20) in Liq. Paraffin while the hydrophilic surfactant (Tween 20) was dissolved in 2.5 ml of distilled water to obtain the aqueous phase. The Ketoconazole was dissolved in Ethanol, and Methyl paraben, Propyl paraben are dissolved in Propylene glycol, then Propylene glycol solution and drug solution are mixed together.

This formed solution was then added in to the aqueous phase. Both the oil and aqueous phase were heated to 70–80°C, followed by the addition of the oil phase into the aqueous phase with constant stirring, until it cooled to room temperature. The obtained emulsion was mixed with the weighed quantity of the gelling agent, in the ratio 1:1, to obtain an elegant gellified emulsion. And added glutaraldehyde in during of mixing of gel and emulsion.

Optimization:

Experimental design: Eight ketoconazole emulgel formulations **(Table 1)** were prepared according to a $2*2^2$ factorial design employing the qualitative factors and levels shown in **Table 2** and **Table 3**. Two factors selected were: Effect of concentration of gelling agent and Effect of emulsifying agent concentration.

TABLE 1: VARIOUS COMPOSITIONS OF EMULGEL FORMULATION

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Carbopol 934 (mg)	500	400	500	400	500	400	500	400
Ketoconazole (mg)	75	75	75	75	75	75	75	75
Minoxidil (mg)	75	75	75	75	75	75	75	75
Liq. Paraffin (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Tween 20 (ml)	0.5	0.5	0.3	0.3	0.5	0.5	0.3	0.3

Span 20 (ml)	0.75	0.75	0.75	0.75	0.45	0.45	0.45	0.45
Propylene glycol (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Ethanol (ml)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Methyl parabene (mg)	10	10	10	10	10	10	10	10
Propyl parabene (mg)	5	5	5	5	5	5	5	5
Glutaraldegyde (ml)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

TABLE 2: FACTOR AND LEVEL FOR 2*2^2 FACTORIAL DESIGN

Factors		Levels
Gelling agent concentration		500 mg (high) 400 mg (low)
Emulsifying agent concentration	Tween 20	0.5 ml (high) 0.3 ml (low)
	Span 20	0.75 ml (high) 0.45 ml (low)

TABLE 3: COMPOSITION OF EMULGEL FORMULATION

Formulation	Composition	
	Gelling agent concentration	Emulsifying agent concentration
F1	+	+
F2	–	+
F3	+	–
F4	–	–
F5	+	+
F6	–	+
F7	+	–
F8	–	–

Characterization of Emulgel:

Physical appearance: The prepared emulgel containing ketoconazole and minoxidil were inspected visually for their color, homogeneity, consistency and phase separation.

Measurement of pH: The pH value of 1% aq. solution of the prepared emulgel was measured by a digital pH meter. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Spreadability: Spreadability is a term expressed to denote the extent of area to which topical preparations readily spreads on application to skin or affected part. Topical formulation should possess good spreadability. The parallel-plate method is the most widely used method for determining and quantifying the spreadability of semisolid preparations. The spreadability is expressed in terms of time in seconds taken by two slides to slip off from cream, placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, resultant the better spreadability. Spreadability was calculated by using the formula.

$$S = \frac{(M \times L)}{T}$$

Where
S= spreadability,
M= Weight tied to upper slide,
L= Length of glass slides and
T= Time taken to separate the slides completely from each other.

Rheological Study: Rheology is the study of flow and deformation of materials under applied forces. The viscosity of different emulgel formulations was determined at 37˚C using a brook field viscometer (Brookfield DV-E viscometer) with spindle 6.

Drug content determination: Drug concentration in emulgel was determined by spectrophotometer after dissolving 1 gm of emulgel in 100 ml of methanol by vortexing and sonication. Absorbance was measured after suitable dilution and filtration at 285 and 255 nm in UV/Visible

spectrophotometer (UV 1700 CE, Shimadzu Corporation, Japan).

***In-vitro* release study:** Franz diffusion cell (with effective area 3.14 cm^2 and 15.5 ml cell volume) was used for the drug release studies. The egg membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared PBS pH 5.5 solution. The receptor chamber was stirred by magnetic stirrer. 1 gm emulgel was applied on to the surface of egg membrane evenly. The samples (1 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV/Visible spectrophotometer at 255 nm and 285 nm after appropriate dilutions. The cumulative amount of drug released across the egg membrane was determined as the function of time. Cumulative corrections were made to obtain the total amount of drug release at each time interval.

***Ex-vivo* release study through rat skin:** Rat skin was prepared according to Scott et al (1986) and stored in phosphate buffer (pH 7.4) in a refrigerator till further use. Rat skin were then placed between the donor and receptor compartments of the Franz diffusion cell (with effective area 3.14 cm^2 and 15.5 ml cell volume), with the dermal side in direct contact with the receptor medium. Optimized formulation F1. 1 gm emulgel was applied on to the surface of the rat skin evenly on donar side. The receptor chamber was filled with freshly prepared PBS pH 5.5 solution. The receptor chamber was stirred by magnetic stirrer at optimized speed. The samples (1 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV/Visible spectrophotometer at 255 nm and 285 nm after appropriate dilutions. Cumulative correction was made to obtain the total amount of drug release at each time interval [19].

Determination of drug deposited in rat skin: Drug deposited in the rat skin was determined by determining the amount of drug remained to be released from rat skin after 24 Hrs and amount of drug released through rat skin in 24 Hrs. Amount of drug remained to be released after 24 Hrs can be determined by dissolving the remained amount of formulation in to 100 ml of methanol by vortexing and sonication. Absorbance was measured after suitable dilution and filtration at 285 and 255 nm in UV/Visible spectrophotometer (UV 1700 CE, Shimadzu Corporation, Japan). Drug remained to be released was calculated using the simultaneous equations. % of drug remained to be released was calculated by using the following formula.

$$Drug\ remained\ to\ be\ release = \left(\frac{C}{T}\right) \times 100$$

Where

C= Practical amount of drug remained in 1 gm formulation after 24 Hrs

T= Theoretical amount of drug in 1 gm of formulation

Thus amount of drug deposited in the rat skin was calculated as

% of drug deposited in ret skin =

total % of drug in 1 gm formulation – (% of drug remained to be released after 24 Hrs + % of drug released through rat skin in 24 Hrs)

RESULTS AND DISCUSSION:

Physical examination: The prepared emulgel formulations were white viscous creamy preparation with a smooth and homogenous appearance.

Measurement of pH: The pH value of all prepared formulations ranged from 5.3 to 5.7, which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Spreadability: The spreadability of various emulgel preparations were measured by parallel plate method. Values are given below in **Fig.1**.

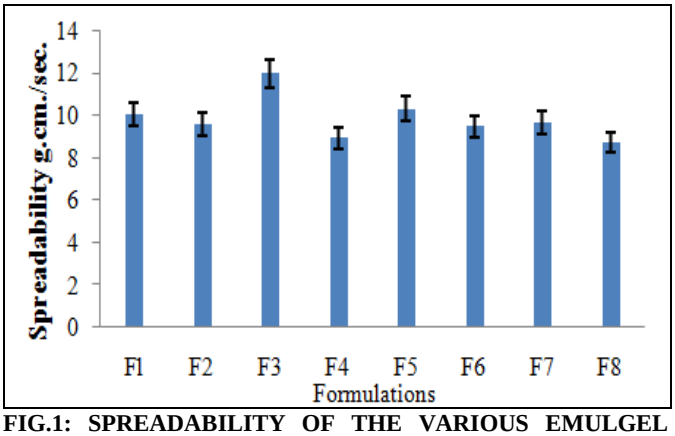

FIG.1: SPREADABILITY OF THE VARIOUS EMULGEL FORMULATIONS

Rheological studies: Measurement of viscosity of emulgels were done with Brookfield viscometer (Brookfield DV-E viscometer). Emulgels were rotated at 1(min) and 100 (max) r.p.m. with spindle 6. At each speed the corresponding dial reading was noted. Obtained viscosities are summarized in **Fig.2.**

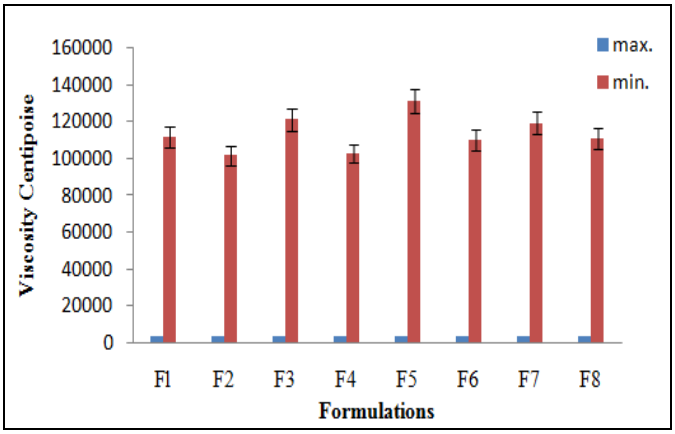

FIG. 2: VISCOSITY OF EMULGEL FORMULATIONS (MEAN ± S.D)

Drug content determination: Drug concentration in emulgel was determined by spectrophotometer. Drug content in emulgel was measured after dissolving 1 gm of emulgel in 100 ml of methanol by vortexing and sonication. Absorbance was measured after suitable dilution and filtration at 285 and 255 nm in UV/Visible spectrophotometer (UV 1700 CE, Shimadzu Corporation, Japan). Drug content of all emulgel formulations are given below in **Fig.3.**

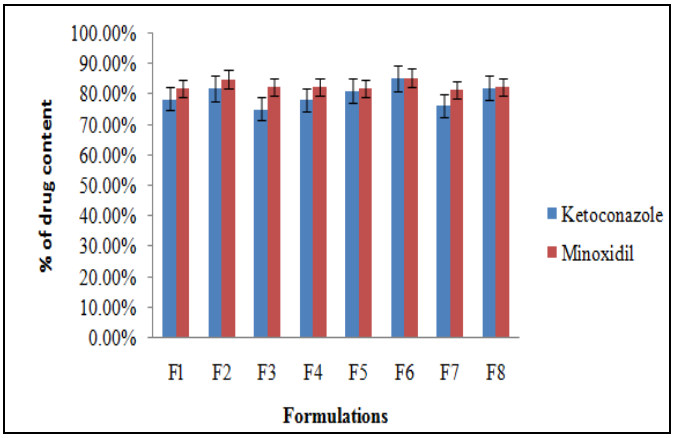

FIG. 3: DRUG CONTENT OF VARIOUS EMULGEL FORMULATIONS (MEAN ± S.D)

***In-vitro* drug release:** *In vitro* release profile of both drugs from various emulgel formulations are represented in **Fig.4.**

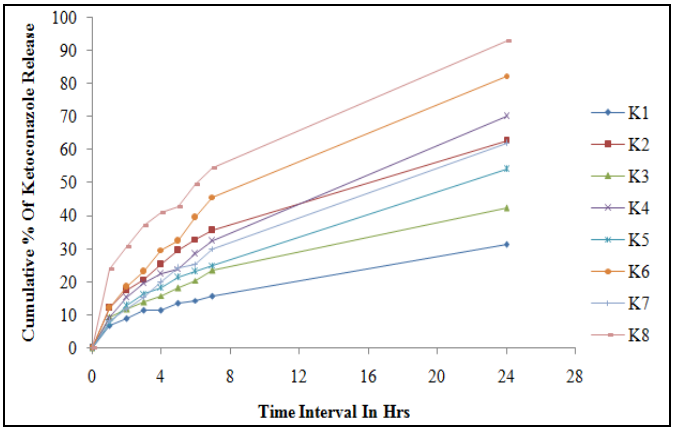

FIG.4: RELEASE PROFILES OF KETOCONAZOLE FROM ITS EMULGEL FORMULATIONS AT 24 HOURS (MEAN ± S.D.)

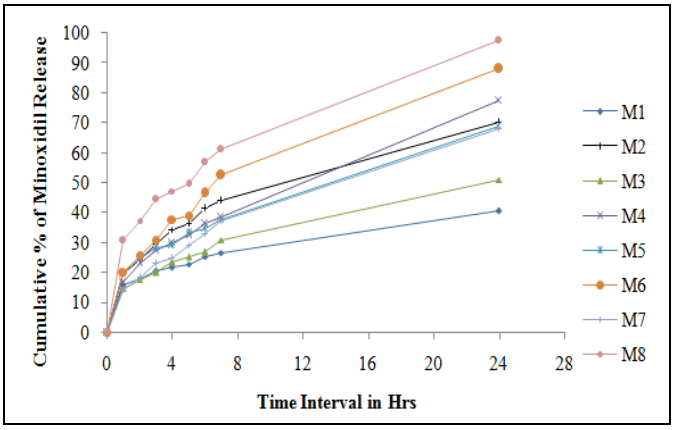

FIG.5*:* RELEASE PROFILES OF MINOXIDIL FROM ITS EMULGEL FORMULATIONS AT 24 HOURS (MEAN ± S.D.)

It was observed that all the formulation had become liquefied and diluted at the end of the experiments, which indicate water diffusion through the membrane. The release of the drugs from its emulsified gel formulation was found in the following ascending order: F1 < F3 < F5 < F7 < F2 < F4 < F6 < F8, Where the amounts Ketoconazole released after 24 hours were 31.39%, 42.34%, 54.04%, 61.79%, 62.56%, 69.89%, 82.23% and 92.65% respectively, and amount of Minoxidil released after 24 hours were 40.66%, 51.05%, 69.02%, 68.06%, 70.01%, 77.26%, 88.01%, 97.63% respectively.

Thus F1 is optimized formulation because of having lowest drug release in circulation and higher drug deposition in skin which is needed for topical action. The lowest drug release was found from the formulation F1. This finding might be due to presence of gelling agent and emulsifying agents in its higher concentrations. Higher concentration of gelling agent might lead to an increased cross

linking in its structure and increased viscosity which hinders penetration of release medium in to formulation and diffusion of drugs from emulgel while higher level of emulsifying agent might reduce hydrophilicity of the formulation thus it further decreases the drug release. Glutaraldehyde was added to formulation to retard the release of drugs. Opposing to formulation F1, formulation F8 showed the highest drug release.

This finding might be due to presence of gelling agent and emulsifying agents in its lower level, low level of emulsifying agent lead to an increase in the hydrophilicity of the emulgel, which in turn facilitates penetration of the release medium into the emulgel. When compared release from formulations F1, F3 and F5, formulation F3 and F5 showed higher drug release than F1 which might be due to lower level of Tween 20/Span 20 in F3 and F5 respectively which increases hydrophilicity of formulations. These finding indicates that effect of span 20 on drug release is more pronounced than the effect of Tween 20. When release from F2 and F3 was compared, F2 showed higher drug release. It might be due to lower level of gelling agent which increases the release but it also contains higher level of Tween 20 than F3 which should decrease the release. Thus these finding indicates that lowering effect of Gelling agent on drug release was more pronounced than the lowering effect of Tween 20. When compared the release from formulation F7 and F4, F4 showed higher drug release than F7. This might be due to lower level of Gelling agent in F4, which is responsible for lowering the drug release but it also contains higher level of Span 20 which should decrease the drug release from F4. Thus from these finding it can be concluded that lowering effect of Gelling agent on drug release is more pronounced than lowering effect of Span 20. Thus effect of different factors on drug release can be concluded as: Gelling agent > Span 20 > Tween 20.

***Ex-vivo* drug release through rat skin and drug deposition:** *Ex-vivo* release profile of drug from optimized formulation F1 are represented in fig 6. Release of emulgel formulation F1 through rat skin showed further retardation in the drug release. It was observed 5.53±0.59 % for ketoconazole and 27.18±0.56 % for minoxidil. This may be due to the thickness of the membranes used. Since the thickness of rat skin membranes is greater it release less drug and retain more, while egg membrane is comparatively thinner and showed higher drug release. 41.62 % ketoconazole and 15.83 % minoxidil was found to be deposited in the rat skin. Deposited ketoconazole in rat skin will help in cure of deep seated dandruff in stratum corneum. The prepared emulgel showed sustained release profile and act as a depot of drugs hence the developed formulation may be used to treat concurrent/chronic dandruff associated with diffused alopecia.

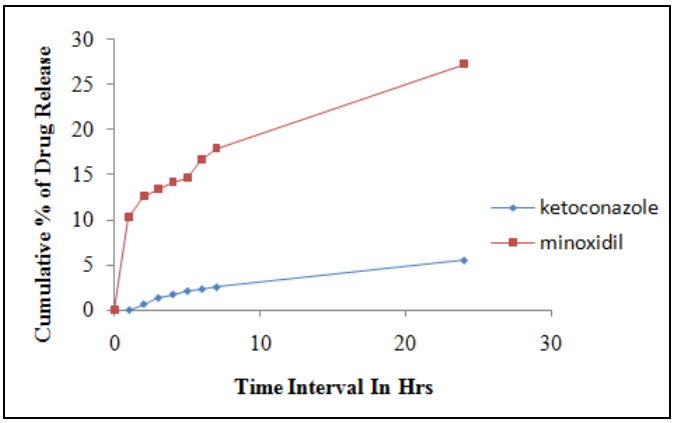

FIG.6: RELEASE PROFILES OF KETOCONAZOLE AND MINOXIDIL FROM F1 AT 24 HOURS (MEAN ± S.D.)

However preclinical studies have not done to study the simultaneous effect of both drug. It is necessary to do preclinical study to obtained the combined effect of ketoconazole and minoxidil for treatment of dandruff and associated diffused alopecia.

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