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NARINGIN A POTENT ANTIOXIDANT USED AS BIOAVAILIBILITY ENHANCER FOR TERBINAFINE HYDROCHLORIDE

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ABSTRACT

The poor bioavailability of drugs has been identified as the single most important challenge in oral drug delivery. Prominent among the factors responsible for this are the oxidative metabolic activity of the intestinal and hepatic cytochrome P450 enzyme family. Naringin and naringenin which are the major phytochemical component of grapefruit juice, a well-known cytochrome P450 3A4 inhibitor and flavone glycoside, is antioxidant in nature and occurs naturally in the pericarp of citrus fruit, and particularly of grapefruit (*Citrus paradisii*) where it is the predominant flavonoid found and is responsible for the bitter taste associated with the fruit. CYP3A4 which is a class of CYP – 450 (microsomal enzyme) is responsible for the oxidative metabolic reaction of various substrates which decreases the bioavailability of drug.

Keywords: Bioavailability, flavone, naringin, cytochrome.

INTRODUCTION

The ability of administered drug to elicit the desired pharmacologic response and reverse or modify a disease condition is the ultimate goal of drug therapy. Serum drug concentration determines the availability of drug molecules at their receptor site. Thus, the rate and extent of drug absorption (bioavailability) from the GIT is an important factor that determines the plasma concentrations of orally administered drug. With a general preference for the oral route of drug administration, first-pass drug metabolism due to oxidation has become one of the most singularly important considerations in drug delivery [1].

With regard to CYP3A4 substrates, the abundance of CYP3A4 in the intestines and the liver makes the oral bioavailability of its substrates extremely poor [2].

Thus, successful inhibition of their pre-systemic activity presents the potential for improved oral bioavailability of drugs [3]. In addition, the required oral dosage may be greatly reduced, thus enhancing patient convenience and compliance. Studies in this area have shown that certain chemical compounds including therapeutic agents, herbal extracts and phytochemicals are capable of inhibiting the metabolic activity of CYP3A4 [4].

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Hepatic enzymes shows Phase 1 Reaction for biotransformation of First pass metabolism i.e Oxidation. Herbal extract which may be antioxidant (Naringin- active Flavanoid of grape fruit) may cause reversible or irreversible inhibition of CYP enzymes and thus prevent biotransformation of substrates. In reversible inhibition, a preferentially lipophilic compound binds tightly within the active site of the enzyme. This prevents binding of other substrate molecules to active hydrophobic regions on the CYP apoprotein or oxygen activationby CYP heme, both actions resulting in transient although sometimes potent inhibition of enzyme activity.

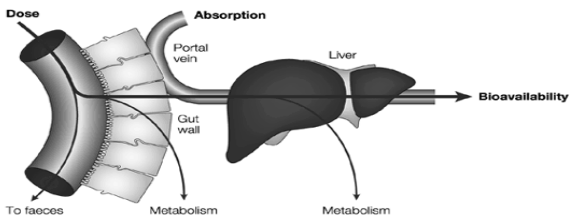


Figure 1: Schematic illustrating the pre-systemic fate of drug administered via the oral route

MECHANISMS OF CYP INHIBITION [5]:

Hepatic enzymes shows Phase 1 Reaction for biotransformation of First pass metabolism i.e

Oxidation. Herbal extract which may be antioxidant (Naringin- active Flavanoid of grape fruit) may cause reversible or irreversible inhibition of CYP enzymes and thus prevent biotransformation of substrates. In reversible inhibition, a preferentially lipophilic compound binds tightly within the active site of the enzyme. This prevents binding of other substrate molecules to active hydrophobic regions on the CYP apoprotein or oxygen activationby CYP heme, both actions resulting in transient although sometimes potent inhibition of enzyme activity.

GRAPE FRUIT

The grapefruit (*Citrus paradisi*) is a subtropical citrus tree known for its sour to semi-sweet fruit [6]. Grapefruit contains a number of polyphenolic compounds, including the flavanone naringin, alongside the two furanocoumarins bergamottin and dihydroxy bergamottin. These inhibit the drug-metabolizing enzyme isoform CYP3A4 predominantly in the small intestine, but at higher doses also inhibit hepatic CYP3A4. It is via inhibition of oxidation of this enzyme that grapefruit increases the effects of a variety of drugs by increasing their bioavailability [7-12]

Figure 2: Role of CYP Enzymes in Hepatic Drug Metabolism

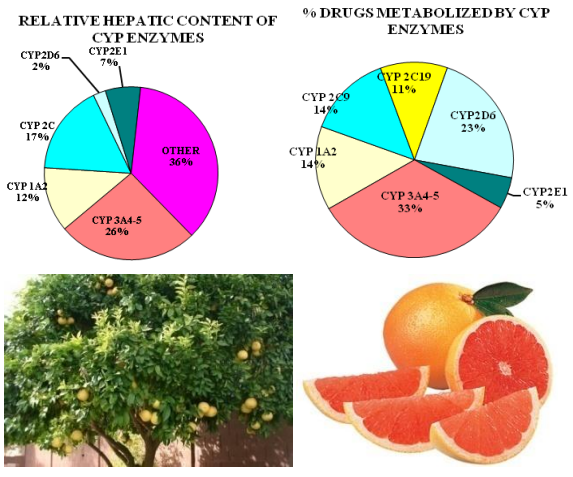
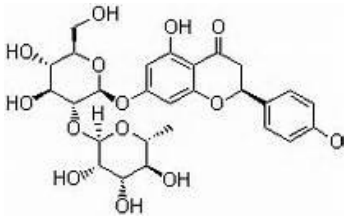


Figure 3 - Image of Grape fruit

NARINGIN- AN ACTIVE FLAVONOID OF GRAPE FRUIT [13, 14]:

Naringin is the major flavonoid glycoside in grapefruit and gives grapefruit juice its bitter taste. It is metabolized to the flavanone naringenin in humans. Both naringin and hesperidins, which are the aglycones of naringin and hesperidin, occur naturally in citrus fruits.

Naringin exerts a variety of pharmacological effects such as antioxidant activity, blood lipid lowering, anticancer activity, and inhibition of selectdrug metabolizing cytochrome P450 enzymes, including CYP3A4 and CYP1A2, which may result in drug - drug interactions in vivo. Ingestion of naringin and related flavonoids can also affect the intestinal absorption of certain drugs, leading to either an increase or decrease in circulating drug levels.



Structural formula of Naringin

MATERIAL AND METHOD:

The material required for the formulation of double layer tablet requires following material:

Lactose monohydrate, Calcium carbonate (CDH, New Delhi), Sodium Starch glycolate (CDH, New Delhi), Micro crystalline cellulose (CDH, New Delhi), Maize Starch (CDH, New Delhi), Poly vinyl pyrollidon k_{30} (CDH, New Delhi), Chitosan (Balaji drugs), Eudragit L-100 (Balaji drugs), Bio extract of Grape fruit (Naringin), Drug.

1. COLLECTION OF GRAPEFRUIT PEELS FOR EXTRACTION OF NARINGIN:

The grapefruit peels were collected from local market of Dehradun, Uttarakhand and it was used for the extraction of naringin (flavonoid).

2. **Extraction of naringin from grapefruit peels [15-18]:**

The extraction of Naringin, the principal flavonoid of Grapefruit rind was done by Soxhlet Extraction method. In this method fresh samples of grapefruit peel were taken. 40.0 gram was grounded in a blender with 100 ml of Ethyl alcohol for 1 minute. The mixture was than filtered and filtrates and residue was divided. The residue was air dried to remove alcohol. The portion of filtrate was placed in a Solvent Extraction flask with 50 ml of ethanol and extraction was carried out for 3 hours. The filtrate was then evaporated at 50 °C, dried at 50 °C and stored at room temperature.

Fig 4: Extraction of naringin from grapefruit peel.

CONFIRMATORY TEST OF NARINGIN:

Test	Inference	Result
5mg of naringin is dissolved in 10ml of ethyl alcohol, 1-2 drop of ferric chloride solution are added.	Brown colour	Brown colour is present.
5mg naringin is dissolved in 5ml of sodium hydroxide solution.	Orange yellow colour	Orange yellow colour is present.

***IN – VITRO* DETERMINATION OF ANTIOXIDANT ACTIVITY OF NARINGIN**:

The CYP3A4 shows oxidative metabolism of drug and the naringin acts as antioxidant which inhibits the oxidative metabolism causes by CYP3A4. The determination of antioxidant activity of naringin is done by using DPPH (2, 2- diphenyl-1- picrylhydrazyl).

QUALITATIVE ANALYSIS: In order to detect the antioxidant activity, a method based on reduction of 2, 2- diphenyl – 1- picrylhydrazyl (DPPH) can be carried out. DPPH is a free radical stable at room temperature, which produces a violet solution in methanol. When the free radical reacts to an antioxidant, its free radical property is lost due to chain breakage and its color changes to light yellow.

The procedure in brief is – extract resolved in solvent is spotted on the silica- gel TLC plates and the chromatogram is developed. The methanolic solution of DPPH (2mg/ml) is prepared. After spoting the extract (naringin) on TLC plates, the plates were immersed in DPPH methanolic solution. The plates were removed allowed for air drying after drying the Vanillin/H_2SO4 reagent is sprayed on the plates as visualizing agent. After sprying the plates were observed under UV-365.The orange Yellow colour indicates presence of Flavonoid (naringin).

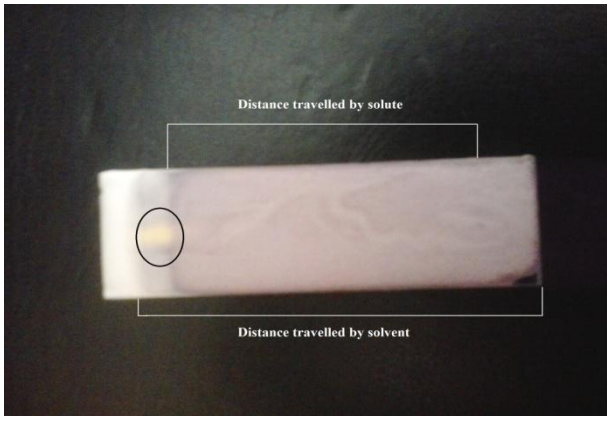


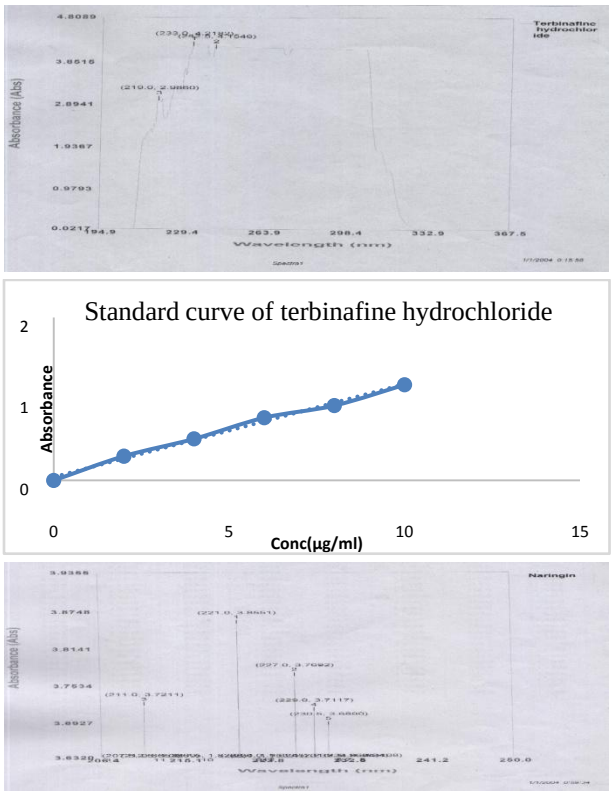
Figure 5: Identification of antioxidant activity of antioxidant (Naringin) on TLC plates

Scanning of Terbinafine hydrochloride by U.V-visible Spectroscopy: Scanning of Terbinafine hydrochloride was determined by making dilution in methanol and observing under U.V- Visible Spectrophotometry in the range 200-400 nm.

It was found out 233 nm.

Standard curve of Terbinafine hydrochloride: the standard curve of terbinafine as obtained by making dilutions in ethanol and then observing under U.V Spectroscopy.

Scanning of Naringin by U.V-Visible Spectroscopy: The scanning of naringin was determined by making dilution in ethanol and observing under U.V- Visible Spectrophotometry in the range 200-400 nm and was found 221 nm



UV scanning report and standard curve of Terbinafine hydrochloride and UV scanning report of naringin

METHADOLOGY:

Formula for the formulation of inner layer (naringin):

For the formulation of inner layer the ingredients are Naringin (100 mg), Lactose monohydrate (300 mg), Chitosan (100 mg), Eudragit L-100 (150 mg) and Poly Vinyl Pyrollidon K_{30} (16 % w/v).

Formulation Methodology: Wet Granulation Method

Reason for the formulation of double layer tablets:

Terbinafine hydrochloride has its absorption window in stomach, so it is necessary to deliver the drug in the stomach, whereas the naringin has its absorption window in small intestine and it is unstable in acidic condition. Hence it is necessary to coat the naringin tablets with enteric coating polymers and deliver it into small intestine whereas the terbinafine hydrochloride tablets will release into stomach only.

All the ingredients were weighed accurately and blended well. Binder solution was prepared in distilled water and added to form granules. Granules were allowed to air dry so that the moisture gets evaporated. Granules were allowed to punch in double layer tablets.

Sr. No	Ingredients	Formulation			
		F1	F2	F3	F4
1.	Drug(Terbinafine Hydrochloride) (mg)	100	100	100	100
2.	Calcium Carbonate (mg)	150	150	150	150
3.	Micro Crystalline Cellulose (mg)	100	150	200	-
4.	Sodium Starch Glycolate (mg)	-	-	-	300
5.	Lactose monohydrate (mg)	300	300	300	300
6.	Tragacanth (w/v)	-	13%	-	-
7.	Poly Vinyl Pyrollidone k_{30} (w/v)	12%	-	-	15 %
8.	Maize Starch paste (w/v)	-	-	13 %	-
9.	Magnesium Stearate	q.s	q.s	q.s	q.s

Table 1: formula used for formulation of outer layer (terbinafine hydrochloride):

EVALUATION PARAMETERS:

Flow properties of granules:

A) Angle of Repose:

The maximum angle possible between the surface of a pile of powder and the horizontal plane Poorly flowing

powders or granulations present many difficulties to the pharmaceutical industry.

B) Bulk Density:

Bulk density is defined as the ratio of mass of the powder and its bulk volume

C) Weight variation test:

20 tablets were weighed and the average weight was calculated. The individual weight was compared with the average weight. The tablet pass the test if not more than two tablets are outside the percentage limit and if no tablet differs by, more than two tablets the percentage limit. The following percent deviation in weight variation is allowed according to U.S.P.

Formulation	Parameters			
	Angle of repose	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's Ratio
F1	26°56´	0.519	0.523	1.007
F2	27° 03´	0.522	0.527	1.0095
F3	26°21´	0.60	0.66	1.1
F4	25°33´	0.503	0.514	1.021

Table 2: Flow properties of Terbinafine hydrochloride granules

Hardness test:

Hardness indicates the ability of tablets to withstand mechanical shocks while handling. The hardness of tablet was determined by using Monsanto Hardness Tester. It was expressed in kg/cm^2.

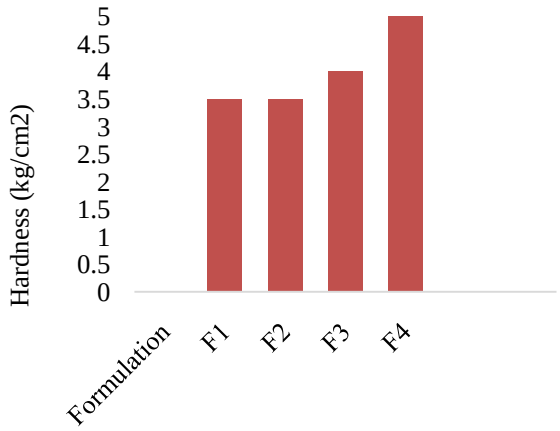


Fig- 5 hardness parameter of different formulations

Disintegration test:

Disintegration is defined as the process of breakdown of tablet into fragments. The disintegration time for uncoated tablet is 5 min. Since we are using two layers hence we have to test both layer i.e uncoated outer layer (Terbinafine hydrochloride) and coated inner layer (Naringin).

***In- vitro* dissolution studies:**

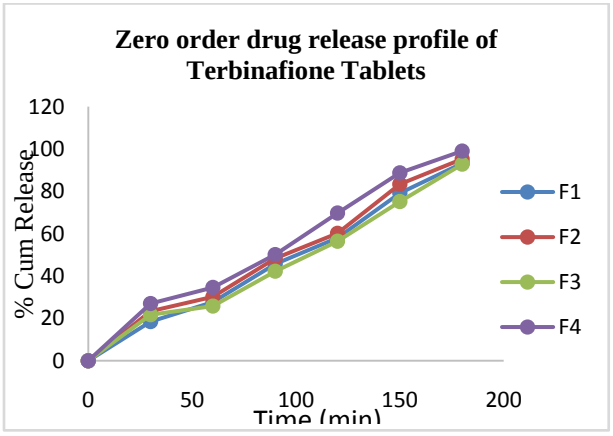
Double layer tablets were subjected to *in – vitro* dissolution studies in simulated gastric and intestinal fluids to assess their ability in providing the desired drug delivery. The temperature was maintained at 37 ± 2 ^{o}C.

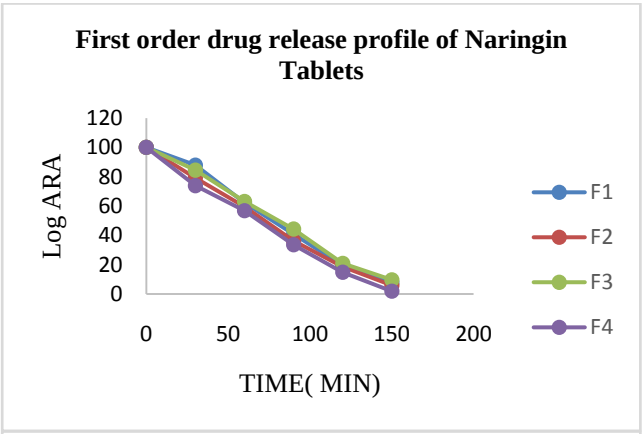
FORMULATION	DISINTEGRATION TIME(min) (Terbinafine hydrochloride)
F1	4 min
F2	4.10 min
F3	5 min
F4	4.3 min

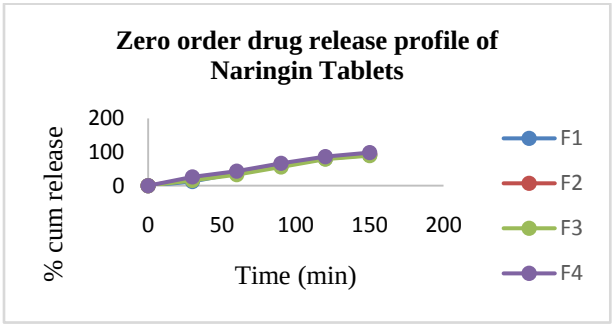
Table 3: Disintegration test of Terbinafine hydrochloride tablet:

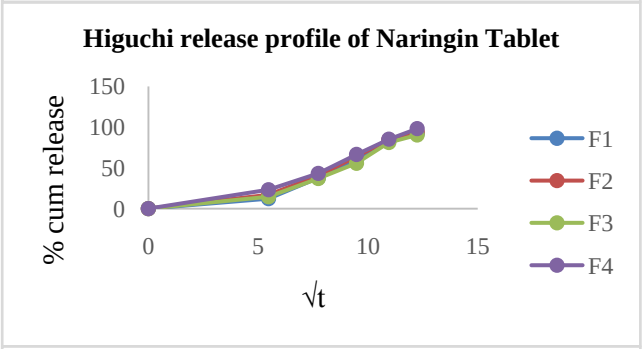
S.n	Formulation and polymer used	Weight of tablet after dip	Disintegration Time
1.	F1 (Eudragit L-100 -12%)	0.254 mg	45 min
2.		0.260mg	1.20 hr
3.		0.305mg	2 hr
4.	F2 (Eudragit S – 100)	0.245	40 min
5.		0.251	1.15 min
6.		0.268	1.45 min
7.	F3 (Ethyl Cellulose-10%)	0.233	1 hr
8.		0.246	1.35 min
9.		0.263	2. 20 min
10.	F4 (Eudragit L- 100 -15%)	0.242	1 hr
11.		0.271	1.30 hr
12.		0.304	2.20 hr

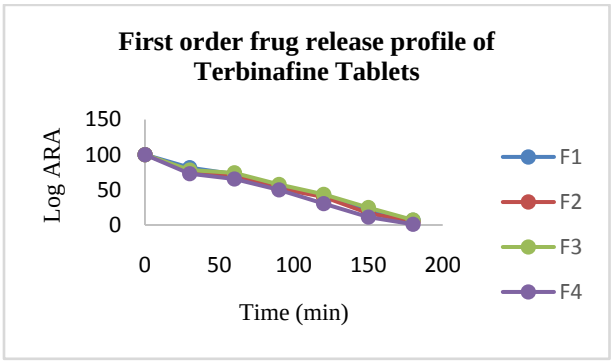
Table 4: Disintegration test of Naringin tablet:

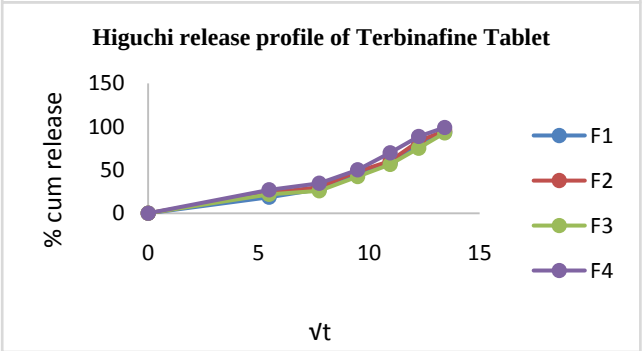












Release kinetics of different formulations after in-vitro release study

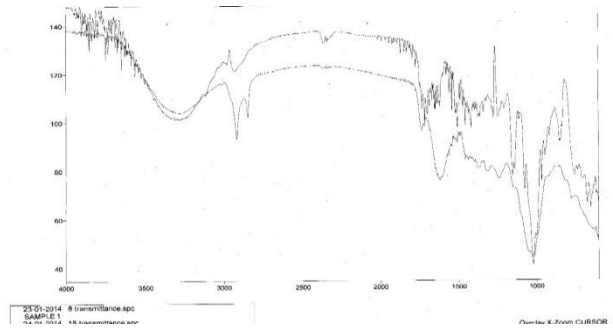


Fig- drug compatibility study through FTIR

CONCLUSION:

The various biochemicals, anatomical and physiological barriers to oral drug absorption and bioavailability have received considerable attention in drug delivery studies. The various approaches aimed at enhancing oral drug delivery were presented in this article. Being responsible for the metabolism and pre-systemic metabolism of greater than 50% of all administered drugs, the CYP enzyme family was identified as the single most important factor influencing oral drug delivery for enhanced bioavailability. The study confirmed that the component flavonoids, the major

phytochemicals found in grapefruit juice are responsible for grapefruit-drug interactions.

The present work involves the formulation and evaluation of Naringin double layer tablet as antioxidant. The method used was wet granulation and various parameters were evaluated. *In – Vitro* studies showed that the formulation F4 was the optimised batch as its N value of Korsmeyer and Peppas is maximum and favours zero order release mechanism and also covers the specification required for formulation. All the evaluation parameter including FTIR have been studied.

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