



## EFFECT OF NANONIZED CHRYSIN ON FEXOFENADINE PHARMACOKINETICS MEDIATED BY P-GLYCOPROTEIN IN RATS

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### ABSTRACT

Chrysin(CHR) is an abundant flavonoid in nature and has been found to possess P-glycoprotein (P-gp) inhibition activity *in vitro*, which may have the potential to alter bioavailability of P-gp substrates. The main objective of this study was to investigate the effect of nanonized chrysin (NCH) on the intestinal absorption and pharmacokinetics of P-gp probe substrate; fexofenadine in rats. NCH was prepared by antisolvent precipitation method and characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and *in vitro* dissolution testing. The influence of CHR and NCH on fexofenadine intestinal transport and permeability were evaluated by *in vitro* non-everted sac and *in situ* single pass intestinal perfusion (SPIP) studies. These findings were further confirmed by an *in vivo* pharmacokinetic study in which fexofenadine was administered (10 mg/kg, p.o) to CHR and NCH (50 mg/kg, p.o.) pretreated (10 days) rats and its plasma concentrations were determined by HPLC analysis. The intestinal transport and apparent permeability (Papp) of fexofenadine were significantly increased in duodenum, jejunum and ileum of CHR and NCH pretreated group as compared with the control. Similarly effective permeability (Peff) of fexofenadine was increased significantly in ileum of CHR and NCH pretreated group as compared with control. Cmax, AUC<sup>0-t</sup> and AUC<sup>0-∞</sup> of fexofenadine were found to be increased in CHR and NCH pretreated groups. Further, the CL/F and Vd/F of fexofenadine were significantly decreased. NCH enhances the oral bioavailability of fexofenadine by inhibition of P-gp mediated drug efflux during the intestinal absorption.

**Keywords:** Chrysin, Fexofenadine, P-glycoprotein, Pharmacokinetics, Nanoparticles.

### 1. INTRODUCTION

Flavonoids are ubiquitous plant specialized metabolites that contain large groups of low-molecular-weight polyphenolic compounds, which present benefits to human health because of their biological properties [1]. Chrysin(CHR) is an abundant flavonoid in nature, and it is also contained by several dietary supplements. It is also called 5, 7-dihydroxyflavone; a flavone contained in several plants (*Passiflora caerulea*, *Passiflora incarnate*, *Oroxylum indicum*, *Matricaria chamomilla*, etc.) and natural products (*Pleurotus ostreatus*, propolis, honey, etc.) [2]. It has various physiological activities including anti-inflammatory, antioxidant, hypoglycemic and anti-aromatase activity [2].

Although the bioactive effects of chrysin are diverse,

there are major challenges limiting their clinical utilization. One of the reasons is that chrysin has poor solubility in water, low physicochemical stability, rapid intestinal and hepatic metabolism, and low cellular uptake which can limit their therapeutic effects [3]. To mitigate these hurdles, use of nano technological approaches can be a promising approach to improve delivery of the compounds to target cells with helping in enhanced bioavailability [4].

The nanoscale modification increases the ratio of surface area to volume and thus may improve solubility. Nanoparticles preparation is roughly divided into two ways, top-down and bottom-up methods. The top-down methods reduce the drug particle size without organic solvent using techniques such as milling (jet mill

and ball mill) and high-pressure homogenization. The bottom-up method uses the particle precipitation from a saturated or unsaturated drug solution, such as solvent evaporation, supercritical fluid, antisolvent precipitation and chemical precipitation [5]. In this study, Nanonization of CHR was done by antisolvent precipitation method using syringe pump (APSP) in order to improve the solubility there by increasing the oral bioavailability of CHR.

Fexofenadine is a nonsedative antihistamine drug (H1-receptor antagonist) and used in the treatment of seasonal allergic rhinitis. It has low oral bioavailability (approximately 33%) in humans due to its low intestinal permeability; P-gp mediated drug efflux. It could be suitable *in vivo* probe to investigate the significance of P-gp-mediated drug interaction without interference of metabolic pathways [6].

Drug efflux transporters such as P-glycoprotein (P-gp; ABCB1), and multidrug resistance-associated proteins 1 and 2 (ABCC1 and ABCC2) are membrane-embedded proteins that limit intracellular concentrations of substrates by pumping them out of the cell. It is well known that flavonoids can inhibit the activities of P-gp and/or drug-metabolizing enzymes [7]. Since chrysin is a P-gp inhibitor and may potentially inhibit efflux transporter responsible for the poor absorption of P-gp substrates, in the present study, we used antisolvent precipitation technique using syringe pump (APSP) method to prepare nanonized chrysin and thereby improve the solubility and bioavailability of chrysin. The main objective of this study was to investigate the effect of nanonized chrysin on the intestinal absorption and pharmacokinetics of P-gp probe substrate; fexofenadine in rats.

## 2. MATERIAL AND METHODS

### 2.1. Chemicals

Fexofenadine and Chrysin were purchased from Sigma-Aldrich (Bangalore, India). Potassium dihydrogen phosphate, absolute ethanol (99.5-99.8%), acetone and acetonitrile (HPLC grade) were obtained from Merck Specialties Pvt. Ltd, (Mumbai, India). Solvents used for quantitative analysis and all other chemicals, reagents which were used in the study, were of analytical grade.

### 2.2. Animals

The study was performed on male wistar rats (150-250g). The rats were kept in polyacrylic cages and maintained under standard laboratory conditions (room temperature 24-27°C and humidity 60-65%). They

were fed with standard pellet diet and water *ad libitum*. Handling and experimentation were conducted in accordance with the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. All *in vivo* animal experimental protocol conducted in this study were approved (06/SPIPS/IAEC/19) by the Institutional Animal Ethical Committee, Kakatiya University, Warangal.

### 2.3. Preparation of Nanonized chrysin (NCH)

NCH was prepared by antisolvent precipitation with a syringe pump (APSP) method. In this method, CHR was dissolved in acetone at concentration of 10 mg/mL. The prepared solution (20 mL) was filled into a syringe and was secured onto a syringe pump. The drug solution was quickly injected at a fixed flow rate (2-10 mL/min) into the deionized water (antisolvent) of a definite volume (solvent/antisolvent ratio-1:15) under magnetic stirring (1,000 rpm). NCH formed from this method was filtered and vacuum dried.

### 2.4. Characterization of NCH

#### 2.4.1. Measurement of Particle Size and Zeta Potential

The average particle size of chrysin nanoparticles was determined by photon correlation spectroscopy with Beckman Coulter, Delsa™ nano, USA at a wavelength of 276 nm. The samples were diluted with water prior to measurements. The zeta potential of chrysin nanoparticles was determined by Malvern Zetasizer Nano® Instrument (Malvern, UK). The zeta potential measurements were performed by using an aqueous dip cell in an automatic mode. Samples were diluted with triple distilled water and placed in capillary measurement cell. The measurements were carried out in triplicate and the average value was calculated.

#### 2.4.1.1. SEM

Surface and shape characteristics of CHR and NCH were evaluated by scanning electron microscopy (SEM) Zeiss EVO 18-EDX special edition instrument compatible with EDX machine. The samples were mounted on aluminum stubs with double sided carbon tape and sputter coated with gold for 4 min before observation. The particle size and texture of nanoparticles was analyzed by using image magnification software compatible with SEM that aids in determining the presence and formation of NCH. Five SEM pictures were used to find the average range of particle diameter.

#### 2.4.1.2. DSC

Differential scanning calorimetry was performed using DSC- 60, Shimadzu instrument. The samples were heated from 40 to 400°C at a heating rate of 10°C/min. The samples (2-3 mg) were heated at a temperature range of 40-400°C. The experiments were performed with a heating rate of 10°C/min under nitrogen at 30 mL/minutes. For analysis, 5 mg of drug i.e. CHR and NCH was used for the study. The resultant thermograms were recorded.

#### 2.4.1.3. In Vitro Dissolution Study

The *In vitro* dissolution of the CHR (5 mg) and NCH (5mg) were determined using the paddle method (Vankel VK 7000 Dissolution Tester, USA) in 100 mL of simulated intestinal fluid (SIF) at pH 6.8 [8]. The paddle rotation was set at 100 rpm and temperature was maintained at 37±0.5°C. The dissolved solution samples of 1 mL were collected at 15, 30, 45, 60, 90, 120 and 180 min of dissolution time. For each sample the dissolution test was performed thrice. The concentration of drug was determined spectrometrically at 348 nm using UV spectrometer (UV-3101PC, Shimadzu, Japan) [9].

### 2.4.2. Study design

#### 2.4.2.1. In vitro non-everted intestinal sac study

An *in vitro* non-everted intestinal sac study was performed according to the previously described methods [10, 11]. Briefly, the rats were divided into three groups control and pretreatment with CHR and NCH, each consisting of six animals. CHR and NCH were administered orally to pretreatment group at a dose of 50 mg/kg for 7 days and other group was kept as control. Both control and pretreatment group rats were sacrificed on 8<sup>th</sup> day by using anesthetic ether, the intestine was surgically removed and flushed with 50 mL of ice cold saline. The small intestine was cut into 3 segments, duodenum, jejunum and ileum of equal length (5 cm). The probe drug (fexofenadine 500 mg/mL) was dissolved in pH 7.4 isotonic Dulbecco's PBS (D-PBS) containing 25 mM glucose. The probe drug solution (1 mL) was filled in the normal sac (mucosal side), and both ends of the sac were ligated tightly. The sac containing probe drug solution was placed in a beaker containing 40 mL of D-PBS, containing 25 mM glucose. The medium was pre-warmed at 37°C and pre-oxygenated with 5% CO<sub>2</sub>/95% O<sub>2</sub> for 15 min, under bubbling with a CO<sub>2</sub>/O<sub>2</sub> mixture gas, the transport of the fexofenadine from

mucosal to serosal direction across the intestinal sacs was measured by sampling the serosal medium periodically for 120 min. The samples of 1 mL were collected from control and pretreatment groups at predetermined time points and stored at -80°C until analysis. The drug transported from mucosal to serosal direction was measured by high performance liquid chromatography (HPLC).

#### Calculation of apparent permeability coefficient (P<sub>app</sub>)

The apparent permeability coefficient (P<sub>app</sub>) of fexofenadine was calculated from the following equation [10]

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{AC_0}$$

Where, dQ/dt is the transport rate of drug in the serosal medium, A is the surface area of the intestinal sacs and C<sub>0</sub> is the initial concentration inside the sacs.

#### 2.4.2.2. In situ intestinal perfusion study

The surgical procedure and the *In situ* single-pass intestinal perfusion (SPIP) study were performed according to the previously reported methods [12, 13]. Briefly, the rats were divided into three groups control and pretreatment with CHR and NCH consisting of six animals each. CHR and NCH were administered orally to pretreatment group at a dose of 50 mg /kg for 7 days and other group was kept as control. Both control and pretreatment group rats were subjected to surgery on 8th day. Rats were subjected to anesthesia by thiopental sodium (50 mg/kg, ip) and they were placed on a hot pad to maintain normal body temperature. A midline incision of 3-4 cm was made on the abdomen of rats and an ileum segment of approximately 8-12 cm was isolated using the ileo-caecal junction as a distal marker. Semi-circular incisions were made at each end of the ileum and the lumen was rinsed with normal saline (37°C) and the both ends were cannulated with polyethylene tubing and ligated by using silk suture. Then, blank perfusion buffer (Phosphate buffer saline, pH 7.4) was first infused for 5 min at a flow rate of 1 mL/min by using Syringe pump (NE- 1600, New Era Syringe Pumps, Inc. NY, USA), followed by perfusion of phosphate buffer saline (pH 7.4) containing fexofenadine (50 mM), propranolol (100 mM) and phenol red (50 mg/mL) at a constant flow rate of 0.2 mL/min for a period of 90 min and perfusate was collected at every 10 min interval. After completion of cannulation, the ileum segment was covered with

isotonic saline-wet gauze (37°C). At the end of perfusion, the length of the ileum segment was measured following the last sample collection. Perfusion samples were collected from control and pretreatment groups at predetermined time points and stored at -80°C until analysis. Fexofenadine concentrations in perfusion samples were measured by HPLC.

#### Phenol red water flux correction

$C_{out}$  (corr) was calculated from the following equation [14]

$$C_{out} \text{ (corr)} = C_{out} \times \left( \frac{\text{Concentration of phenol red in CPR}_{in}}{\text{Concentration of phenol red in CPR}_{out}} \right)$$

Where  $C_{out}$  (corr) is corrected outlet concentration of the drug,  $C_{out}$  is outlet concentration of the drug,  $CPR_{in}$  is concentration of phenol red entering the intestinal segment and  $CPR_{out}$  is concentration of phenol red exiting the intestinal segment.

#### Calculation of effective permeability coefficient ( $P_{eff}$ )

It is estimated from the steady state concentration of compounds in the perfusate samples. The steady state was reached at 30-40 min after the beginning of the experiment and it is considered to be attainable when the concentration level of phenol red (non absorbable marker) is stable. The steady state effective permeability is calculated using the following equation [12].

$$P_{eff} = \left\{ \frac{-Q \times \ln(C_{out}/C_{in})}{2\pi rL} \right\}$$

Where Q is perfusion flow rate (0.2 mL/min),  $C_{out}$  is corrected outlet concentration of the drug,  $C_{in}$  is inlet drug concentration, r is radius of the rat small intestine (0.18 cm) and L is the length of the perfused intestinal segment (10 cm).

#### 2.4.2.3. In vivo pharmacokinetic study

Rats were fasted overnight before oral dose administration and approximately 3 h post-dose. The rats were divided into three groups of control and pretreatment with CHR and NCH consisting of six animals each. CHR and NCH were administered orally to pretreatment group at a dose of 50 mg/kg for 7 days and other group was kept as control. Fexofenadine at a dose of 10 mg/kg was administered orally to both control and pretreatment groups on 8th day. Under ether anesthesia, blood samples (approximately 0.25 mL) were collected from the retro-orbital plexus into heparinized micro centrifuge tubes prior to dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 post-dose and plasma was obtained by centrifuging the blood samples at 13,000 rpm for 15 min and stored at -80°C until HPLC analysis.

HPLC analysis of fexofenadine and propranolol Shimadzu HPLC system equipped with a LC-20AD pump and SPD 20A UV visible detector and RP C18 column (Phenomenex Luna, 250 mm - 4.6 mm ID, particle size 5 mm) was used for the analysis of samples. For fexofenadine, the mobile phase used was a mixture of 0.1 M triethylamine: acetonitrile: methanol (61:19.5:19.5, v/v, pH 4.4 adjusted with phosphoric acid) and the elution was monitored at 195 nm at a flow rate of 1.0 mL/min [6]. In case of propranolol, the mobile phase was used a mixture of methanol: 0.05 mM  $KH_2PO_4$  (55:45, v/v, pH 6.0 adjusted with potassium hydroxide) and the elution was monitored at 227 nm at a flow rate of 1.0 mL/min [15]. All the *in vitro*, *in situ* perfusion and plasma samples were extracted using a simple protein precipitation method by adding acetonitrile (200 mL) to samples (100 mL). Samples were vortexed for 2 min and centrifuged at 13,000 rpm for 15 min. The resultant clean supernatant (20 mL) was injected and analyzed using HPLC method. The limit of detection was 0.01 mg/mL and the assay range used was 0.01-10 mg/mL. The average recovery of the drug was 88.98%. The intra-day and inter-day coefficients of variation for the low and high quality control samples were less than 15%.

#### Spectrophotometric analysis of phenol red

Phenol red is having characteristic red colour in phosphate buffer pH (7.4) and it was measured by spectrophotometric method at 560 nm [15].

#### Pharmacokinetic analysis

Pharmacokinetic parameters were computed by non-compartmental model using Phoenix WinNonlin version 6.2 software (Certara, Pharsight Corporation, USA). The plasma fexofenadine concentration versus time plots were used to estimate the peak plasma concentration ( $C_{max}$ ), time to reach peak plasma concentration ( $T_{max}$ ), area under the concentration time curve to the last sampling point ( $AUC_{0-t}$ ), area under the concentration time curve to the infinity ( $AUC_{0-1}$ ), half-life ( $T_{1/2}$ ), elimination rate constant ( $K_{el}$ ), clearance ( $CL/F$ ) and volume of distribution ( $V_d$ ).

#### 2.4.3. Statistical analysis

All mean values are presented along with their standard deviation (mean SD). Statistical analysis was performed using Student's unpaired t-test and analysis of variance (ANOVA) using Graph Pad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA) at significance level of  $p < 0.05$ .

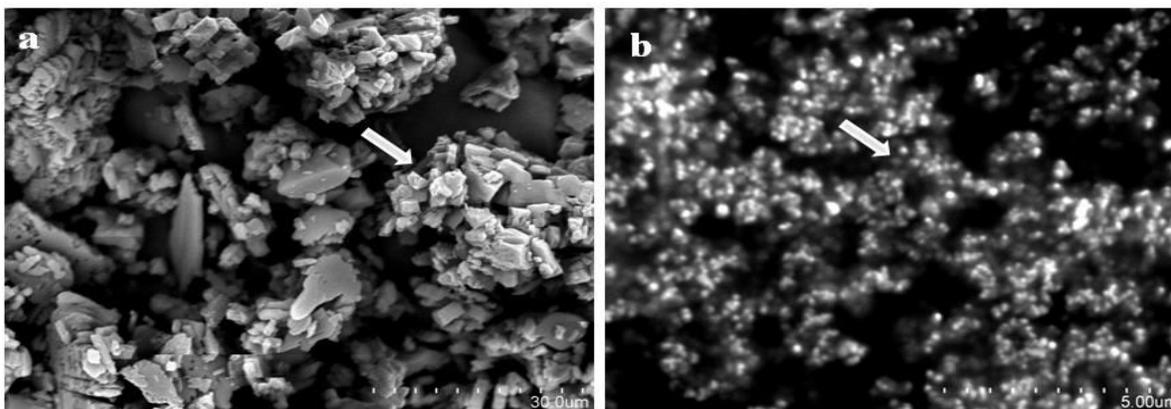
### 3. RESULTS

#### 3.1. Characterization of NCH

##### 3.1.1. SEM and zeta potential analysis

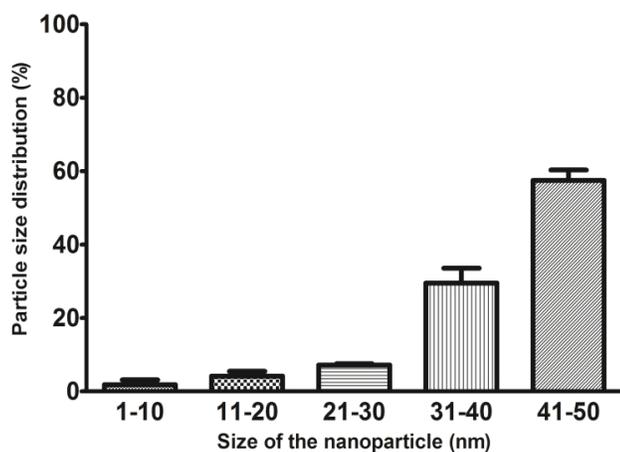
SEM imaging of the CHR and NCH (Fig.1a, Fig.1b.) shows the morphological characteristics of the CHR. As observed, CHR exhibited much larger particles of irregular shapes and lacking size uniformity. In contrast, NCH was regular, spherical in shape and more uniform

in size and shape. Chrysin particles were found to be in the range of 1-3.5  $\mu\text{m}$ . After nanonization, the majority of particles were found to be in the range of 40-50 nm (Fig.1c.). The zeta potential of NCH was observed to be in the range of -33.5 to -35.5 MV. A negative zeta value indicated the anionic nature of the drug suggesting an enhanced physical stability of NCH.



a) SEM image indicating the morphology of chrysin before nanonization (magnification, 30  $\mu\text{m}$ ), b) SEM image indicating the morphological changes in chrysin after nanonization (nanonized chrysin) (magnification, 5  $\mu\text{m}$ )

**Fig. 1: Scanning electron microscopic images of chrysin and nanonized chrysin prepared by APSP**



**Fig. 1c: Particle size distribution of nanonized chrysin**

##### 3.1.2. DSC

Thermograms of CHR and NCH were recorded and are shown in fig. 2. CHR showed a sharp melting endothermic peak at 295.8°C, and a melting enthalpy of fusion 89.98 J/g, whereas NCH showed melting endothermic peak at 291.8°C but the melting enthalpy of fusion was lower 45.1 J/g than that of the CHR indicating a decrease in crystallinity. The enthalpy of fusion is proportional to the degree of crystallinity in the

samples. Drugs with the lower crystallinity often result in higher dissolution and thus enhance the bioavailability. The decline in crystallinity of chrysin nanoparticles can be proposed to enhance its solubility and bioavailability.

##### 3.1.3. In vitro dissolution studies

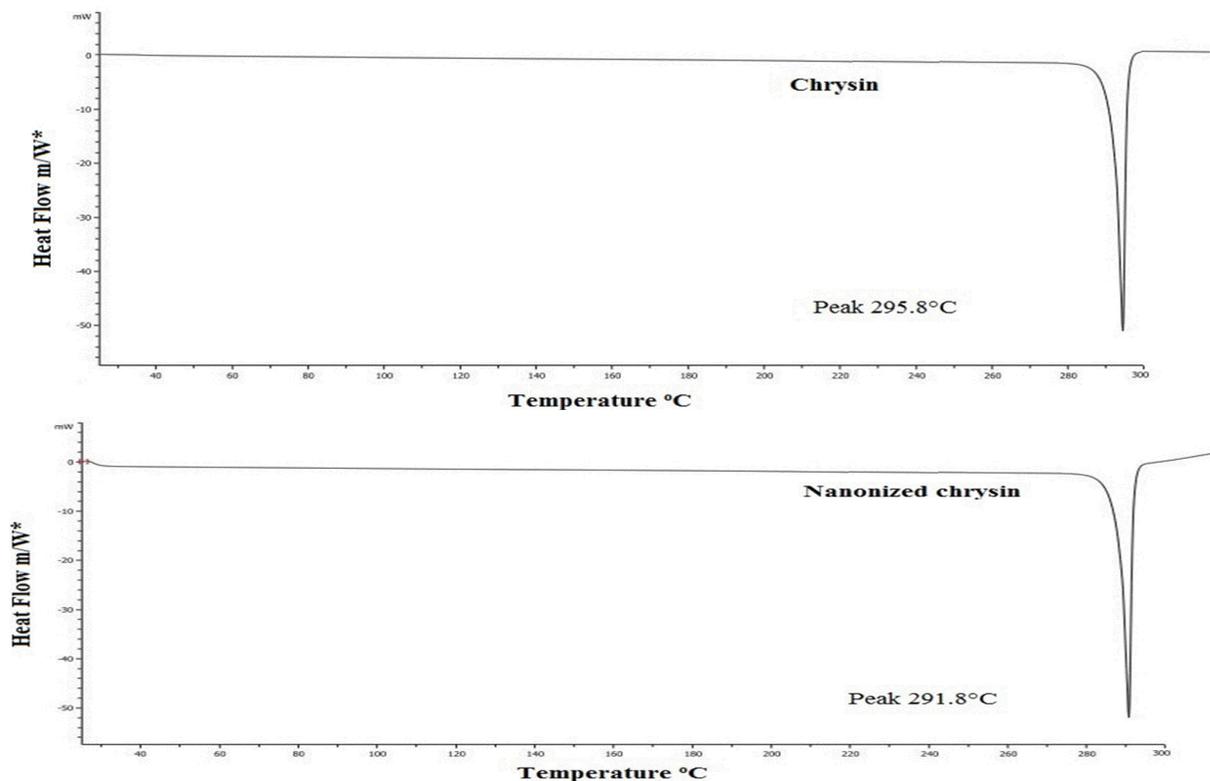
Fig.3. depicts that 94% of NCH was found to be dissolved at 180 min on the other hand, only 50% of CHR was dissolved in the same period. It was observed that NCH reached 50% dissolution in 45 min; whereas CHR did not reach even 50% dissolution at 180 min.

#### 3.2. Effect of CHR and NCH on intestinal transport of fexofenadine

The present study involves the determination of fexofenadine intestinal transport and apparent permeability in control and pretreated rats. Pretreatment with CHR (50 mg/kg, p.o.) for 7 days resulted in a significant ( $p < 0.05$ ) increase in mean cumulative concentrations (from mucosal to serosal direction) of fexofenadine from  $2.23 \pm 0.67$  to  $6.95 \pm 0.69$   $\mu\text{g}$  in duodenum;  $2.53 \pm 0.56$  to  $7.58 \pm 0.78$   $\mu\text{g}$  in jejunum; and  $3.21 \pm 0.43$  to  $8.56 \pm 0.83$   $\mu\text{g}$  in ileum. In addition, pretreatment with NCH (50 mg/kg,

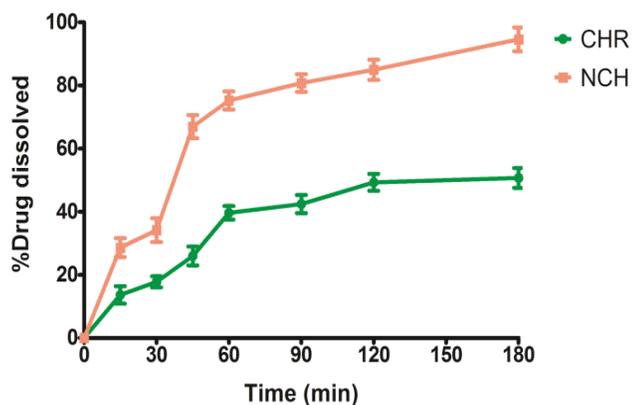
p.o.) for 7 days resulted in a significant ( $p < 0.05$ ) increase in mean cumulative concentrations (from mucosal to serosal direction) of fexofenadine from  $2.23$

$\pm 0.67$  to  $10.32 \pm 0.66$   $\mu\text{g}$  in duodenum;  $2.53 \pm 0.56$  to  $11.24 \pm 0.58$   $\mu\text{g}$  in jejunum; and  $3.21 \pm 0.43$  to  $13.45 \pm 0.72$   $\mu\text{g}$  in ileum (Fig. 4).



a) chrysin showed sharp melting endothermic peak at  $295.8^{\circ}\text{C}$ , and a melting enthalpy of fusion  $89.98$   $\text{J/g}$ , b) nanonized chrysin showed melting endothermic peak at  $291.8^{\circ}\text{C}$ , and a melting enthalpy of fusion  $45.1$   $\text{J/g}$  indicating reduced crystallinity

**Fig. 2: DSC thermograms**



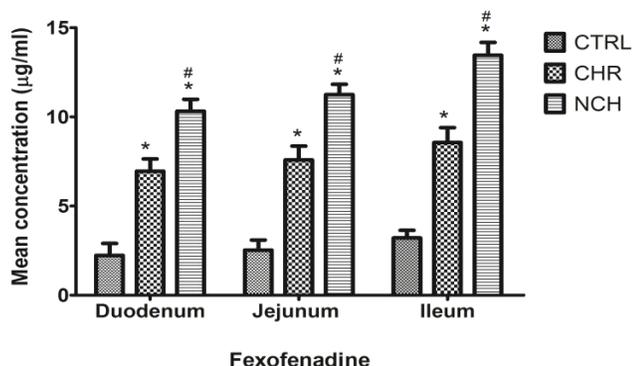
**Fig. 3: Dissolution profile of chrysin and nanonized chrysin, indicating that enhanced solubility of nanonized chrysin**

The transport of fexofenadine was found to be increased by 3.1, 3, and 2.7-fold in duodenum, jejunum and ileum respectively in CHR pretreated group as

compared with that of control group. Further, the transport of fexofenadine was found to be increased by 4.6, 4.4, and 4.2-fold in duodenum, jejunum and ileum respectively in NCH pretreated group as compared with that of control group. The transport of fexofenadine was increased from duodenum to ileum.

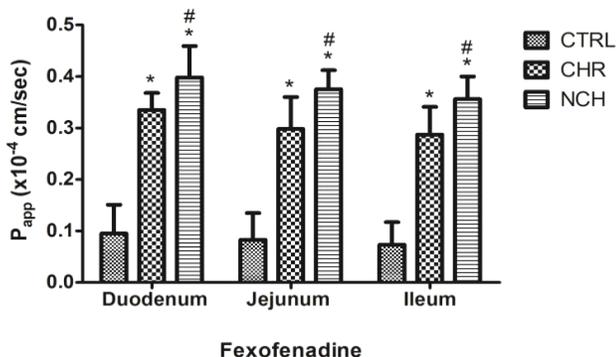
As shown in Fig. 5, pretreatment with CHR resulted in a significant ( $p < 0.05$ ) increase in  $P_{app}$  of fexofenadine from  $(0.095 \pm 0.056) 10^{-4}$   $\text{cm/s}$  to  $(0.335 \pm 0.033) 10^{-4}$   $\text{cm/s}$  in duodenum;  $(0.083 \pm 0.052) 10^{-4}$   $\text{cm/s}$  to  $(0.298 \pm 0.062) 10^{-4}$   $\text{cm/s}$  in jejunum;  $(0.073 \pm 0.044) 10^{-4}$   $\text{cm/s}$  to  $(0.287 \pm 0.054) 10^{-4}$   $\text{cm/s}$  in ileum. In addition, pretreatment with NCH resulted in a significant ( $p < 0.05$ ) increase in  $P_{app}$  of fexofenadine from  $(0.095 \pm 0.056) 10^{-4}$   $\text{cm/s}$  to  $(0.432 \pm 0.061) 10^{-4}$   $\text{cm/s}$  in duodenum;  $(0.083 \pm 0.052) 10^{-4}$   $\text{cm/s}$  to  $(0.412 \pm 0.037) 10^{-4}$   $\text{cm/s}$  in jejunum;  $(0.073 \pm 0.044) 10^{-4}$   $\text{cm/s}$  to  $(0.4 \pm 0.044) 10^{-4}$   $\text{cm/s}$  in ileum. The apparent permeability of fexofenadine in duodenum, jejunum and ileum was found to be

increased by 3.5, 3.6, and 3.9 -fold respectively in CHR pretreated group as compared to control group. Further, the apparent permeability of fexofenadine in duodenum, jejunum and ileum was found to be increased by 4.2, 4.5, and 4.8 -fold respectively in NCH pretreated group as compared to control group. The increased intestinal transport and Papp of fexofenadine were more prominent in NCH pretreated group than the CHR pretreated group.



Control group administered with fexofenadine alone and Pretreatment groups administered with chrysin and nanonized chrysin for 7 days followed by fexofenadine on 8th day. Data are represented as mean  $\pm$  SD ( $n = 6$ ). \*Significant difference ( $p < 0.05$ ) in comparison with the control. # Significant difference ( $p < 0.05$ ) in comparison with the chrysin pretreated groups.

**Fig. 4: Effect of chrysin and nanonized chrysin on intestinal transport of fexofenadine in duodenum, jejunum and ileum of Wistar rats.**

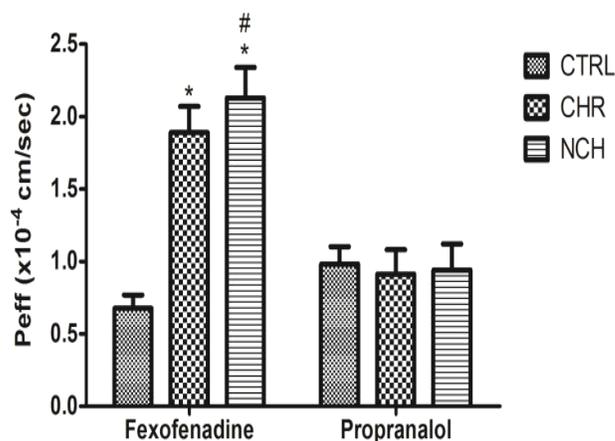


Control group administered with fexofenadine alone and Pretreatment groups administered with chrysin and nanonized chrysin for 7 days followed by fexofenadine on 8th day. Data are represented as mean  $\pm$  SD ( $n = 6$ ). \*Significant difference ( $p < 0.05$ ) in comparison with the control. # Significant difference ( $p < 0.05$ ) in comparison with the chrysin pretreated groups.

**Fig. 5: Effect of chrysin and nanonized chrysin on apparent permeability of fexofenadine in duodenum, jejunum and ileum of Wistar rats.**

### 3.3. Effect of CHR and NCH on intestinal permeability of fexofenadine

Fexofenadine and propranolol intestinal effective permeability (Peff) were determined in rat ileum segment using single pass intestinal perfusion technique. Effective permeability values were calculated from the steady-state concentrations of compounds in the perfusate collected from the outlet. As shown in Fig. 6, pretreatment with CHR for 7 days resulted in a significant ( $p < 0.05$ ) increase in effective permeability of fexofenadine from  $(0.670 \pm 0.090) 10^{-4}$  cm/s to  $(1.890 \pm 0.180) 10^{-4}$  cm/s. In addition, pretreatment with NCH for 7 days resulted in a significant ( $p < 0.05$ ) increase in effective permeability of fexofenadine from  $(0.670 \pm 0.090) 10^{-4}$  cm/s to  $(2.130 \pm 0.210) 10^{-4}$  cm/s while there was no significant change in effective permeability of propranolol and it was found to be  $(0.982 \pm 0.120) 10^{-4}$  cm/s,  $(0.912 \pm 0.170) 10^{-4}$  cm/s and  $(0.940 \pm 0.180)$  in control and pretreated (CHR, NCH) groups respectively. The increase in effective permeability of fexofenadine in ileum was found to be 2.8, 3.2 -fold in pretreated (CHR, NCH) groups as compared with that of control group. Propranolol was shown to have no interaction with P-gp as it is a highly permeable marker.

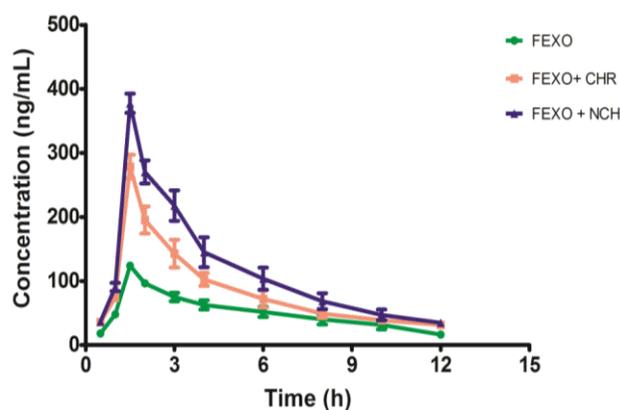


Control group administered with fexofenadine alone and Pretreatment groups administered with chrysin and nanonized chrysin for 7 days followed by fexofenadine on 8th day. Data are represented as mean  $\pm$  SD ( $n = 6$ ). \*Significant difference ( $p < 0.05$ ) in comparison with the control. # Significant difference ( $p < 0.05$ ) in comparison with the chrysin pretreated groups.

**Fig. 6: Effect of chrysin and nanonized chrysin on effective permeability of fexofenadine and propranolol in ileum of Wistar rats**

### 3.4. Effect of CHR and NCH on pharmacokinetics of fexofenadine

The effects of CHR and NCH (50 mg/kg, p.o.) on the plasma concentration-time plots of orally administered fexofenadine (10 mg/kg, p.o.) were characterized and depicted in Fig. 7, and the pharmacokinetic parameters were summarized in Table 1. CHR and NCH pretreatment for 10 days significantly ( $p < 0.05$ ) altered the pharmacokinetics of oral fexofenadine when compared with the control group (fexofenadine alone). The increase in  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  of fexofenadine were found to be 2.2, 1.6 and 1.8; 3.1, 2.3 and 2.3- fold respectively in CHR and NCH pretreated groups. Further, the  $CL/F$  and  $Vd/F$  of fexofenadine were significantly decreased while there was no significant change was observed in  $T_{max}$  of fexofenadine in CHR and NCH pretreated group when compared with control group.



Control group administered with fexofenadine alone and Pretreatment groups administered with chrysin and nanonized chrysin for 7 days followed by fexofenadine on 8th day. Data are represented as mean  $\pm$  SD ( $n = 6$ ). \*Significant difference ( $p < 0.05$ ) in comparison with the control.

**Fig. 7: Plasma drug concentration-time plots of Fexofenadine**

**Table 1: Pharmacokinetic parameters of fexofenadine in control, chrysin and nanonized chrysin pretreated group rats after oral administration of 10 mg/kg**

PK parameters	Fexofenadine	Fexofenadine +CHR	Fexofenadine + NCH
$C_{max}$ (ng/mL)	124.02 $\pm$ 5.74	278.73 $\pm$ 18.58*	377.54 $\pm$ 14.83*#
$T_{max}$ (h)	1.50 $\pm$ 00	1.50 $\pm$ 00	1.50 $\pm$ 00
$AUC_{0-t}$ (h ng/ml)	596.66 $\pm$ 62.19	981.12 $\pm$ 83.57*	1352.53 $\pm$ 144.19*#
$AUC_{0-\infty}$ (h ng/ml)	689.68 $\pm$ 45.05	1249.68 $\pm$ 104.36*	1562.15 $\pm$ 151.11*#
$Ke$ (h <sup>-1</sup> )	0.21 $\pm$ 0.08	0.13 $\pm$ 0.03	0.11 $\pm$ 0.03
$t_{1/2}$ (h)	3.59 $\pm$ 1.07	5.81 $\pm$ 1.45	6.16 $\pm$ 0.80
$CL/F$ (L/h)	14.55 $\pm$ 0.9	8.05 $\pm$ 0.63	6.45 $\pm$ 0.57*#
$Vd/F$ (L)	76.65 $\pm$ 23.87	67.82 $\pm$ 17.13	40.10 $\pm$ 8.38*#

Data values are presented as mean  $\pm$ SD ( $n = 6$ ). \* Significant difference ( $p < 0.05$ ) in comparison with the control. # Significant difference ( $p < 0.05$ ) in comparison with the control.

## 4. DISCUSSION

The drug efflux transporter P-glycoprotein, which is encoded by the Multidrug Resistance Protein-1 (MDR1), is a membrane transport protein expressed in the intestine, liver, kidney, placenta, and blood-brain barrier. P-glycoprotein mediates the cellular elimination of a wide variety of chemically unrelated drugs, including verapamil, digoxin, tacrolimus, clarithromycin, fexofenadine, and saquinavir [16]. Natural products such as juices, fruits, vegetables and herbal products in the form of ayurvedic medicine have been reported in several studies to potentially cause many drug interactions affecting drug absorption mediated by transporters [17]. Therefore, more preclinical investigations on the drug-phytochemical

interactions should be performed to prevent potential adverse reactions or utilize those interactions for a therapeutic benefit.

The present study evaluated the effects of NCH, on the pharmacokinetics of fexofenadine in rats to examine a potential pharmacokinetic interaction between NCH and fexofenadine via the inhibition of P-gp. In this study, fexofenadine was used as a putative P-gp probe substrate to investigate the possible interaction with NCH in rats by using an *in vitro*, *in situ* and *in vivo* model. Moreover, fexofenadine pharmacokinetics primarily depends on the P-gp activity without interference of metabolic pathways [6]. Although, chrysin possesses wide range of therapeutic effects, its poor water solubility limited its biocompatibility,

bioavailability and biomedical applications, indicating an important problem for cancer treatment because of quick metabolism, low absorption and rapid systemic elimination. One of the approaches to overcome this problem is the use of nanoparticles [18].

Particle size and zeta-potential are significant factors since they directly impact the stability, biodistribution and cellular uptake of nanoparticles. Reduction in the particle size of active ingredients to nanoparticle size has shown improvement in solubility and bioavailability [18]. In the present study, the particle size of chrysin made smaller and uniform by antisolvent precipitation method. The size range of NCH was observed below 100 nm using scanning electron microscope and zetasizer.

It has been reported that P-gp (*mdr1a*) mRNA expression is significantly increased from duodenum to ileum, in the order ileum > jejunum > duodenum suggesting that higher P-gp expression was found in ileum than jejunum and duodenum [19]. The non-everted sac model was originally used to evaluate drug transport mechanisms [20]. Genty et al. [21] stated that the passive permeability of actively transported molecules can be determined through non-everted rat gut sacs. Hence, in the present investigation, non-everted intestinal sac study was performed to determine the role of P-gp in the intestinal transport and apparent permeability of fexofenadine. The transport of fexofenadine was significantly increased in duodenum, jejunum and ileum sacs of NCH pretreated and CHR pretreated group rats when compared with the fexofenadine alone group rats. Similarly, Papp of fexofenadine was significantly increased in duodenum, jejunum and ileum sacs of NCH pretreated and CHR pretreated group rats in comparison with that of fexofenadine control. The transport and Papp of fexofenadine was significantly ( $p < 0.05$ ) increased in NCH pretreated group rats when compared with the CHR pretreated group rats.

Further, in order to support the role of P-gp inhibition involved in intestinal transport of fexofenadine, *in situ* perfusion studies were performed in ileum of rats. These techniques maintain an intact blood supply to the intestine and can be used to estimate the impact of clearance pathways such as enzymes and transporters present in the gut. Therefore, permeability studies in rat ileum would provide a more meaningful forecast on the functional role of P-gp [22]. In the present study, SPIP was performed in ileum segment of rats to investigate the functional role of P-gp in intestinal permeability of

fexofenadine. The Peff of fexofenadine was significantly increased in ileum of pretreated (CHR, NCH) groups as compared with that of control group. The Peff of fexofenadine was significantly ( $p < 0.05$ ) increased in NCH pretreated group rats when compared with the CHR pretreated group rats.

In addition, in this study the effect CHR and NCH on the pharmacokinetics of fexofenadine was investigated in rats. Pretreatment with CHR and NCH for 7 days enhanced the oral bioavailability of fexofenadine. The AUC and Cmax were significantly increased in rats pretreated with CHR and NCH when compared with control group. Animals pre-treated with NCH showed significant improvement in Cmax and AUC of orally administered fexofenadine compared with animals pre-treated with CHR. In addition, pretreatment with CHR and NCH resulted in a significant decrease in CL/F of fexofenadine while there was no significant change found in tmax of fexofenadine compared with fexofenadine alone group. Decreased CL/F, kel and increased t1/2 values indicating the inhibition of elimination of fexofenadine upon CHR and NCH pretreatment.

These findings provide *in vivo* evidence that NCH might have increased the bioavailability of fexofenadine via the inhibition of P-gp mediated drug efflux during the absorption phase in the intestine. Moreover, the results obtained in our study were comparable to previous reports where piperine [6], diosmin [23] substantially enhanced the bioavailability of fexofenadine through the inhibition of P-gp mediated efflux in intestine of rats.

We have limited data regarding the pharmacokinetics and drug interactions of chrysin in humans; however, animal experiments showed that chrysin can influence the pharmacokinetics of different compounds (e.g., caffeine, nitrofurantoin, and paracetamol) due to its interactions with CYP enzymes and BCRP [24, 7, 25, 26]. Given that a large number of drugs are substrates for P-gp, the concomitant use of chrysin or chrysin containing dietary supplements may provide some therapeutic benefit to improve the pharmacokinetics of poorly absorbable P-gp substrates.

## 5. CONCLUSION

Nanonization is a novel approach to enhance solubility of CHR there by its oral bioavailability. In the present experimental investigation, NCH increased the systemic exposure of fexofenadine might be due to inhibition of P-gp mediated drug efflux during the intestinal absorption.

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### Conflict of interest

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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