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Fast dissolving oral films: An approach to co-load and deliver the atorvastatin and ezetimibe for better therapeutic response

Meshal Alshamrani[1], Iqra Mubeen[2], Muhammad Hashir Iqbal[7], Muhammad Farooq[2*], Muhammad Zaman[4*], Mueen Ahmad Chaudhry[3], Omer Salman Qureshi[5], Muhammad Hammad Butt[4], Ahmad Salawi[1], Muhammad Rizwan Khan[6] and Yosif Almoshari[1]

[1]Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, Saudi Arabia
[2]Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan
[3]Allied College of Health Sciences, Multan, Pakistan
[4]Faculty of Pharmacy, University of Central Punjab, Lahore, Pakistan
[5]Department of Pharmacy, Forman Christian College, Lahore, Pakistan
[6]Imran Idrees College of Pharmacy, Sialkot, Pakistan
[7]District Headquarter Teaching Hospital Sargodha, Sargodha, Pakistan

Abstract: Atherosclerotic patients suffering with acute coronary disease are lying at high risk. This life-threatening problem can be curtailed by using statins e.g., ezetimibe (EZT), atorvastatin calcium (ATC). In this study, co-loaded Fast Dissolving oral films (FDOFs), of ATC-EZT with HPMC E5 prepared by solvent evaporation method. Prepared FDOFs were evaluated for physicochemical, thermal and mechanical properties. *In-vivo* animal studies were performed on albino rats against diet induced hyperlipidemia. Prepared FDOFs have rapid DT; 27sec, TDT >2min and *in-vitro* drug release 97% in a min. In DSC, FTIR and XRD analysis, prepared films were chemically compatible and no chemical interaction of drugs and excipient was found. In kinetic modeling, it was observed their EZT exhibited lowest R^2 value for zero order kinetic, and best fit model was first order kinetic (n, 0.9823). The korsmeyer peppas model films (n, 0.016) indicate fickian type drug diffusion. The groups treated with marketed suspension of drug and FODPs were compared with normal group and high fats diet group. Study reviled that combination FDOPs of both ATC/EZT significantly reduce hyperlipemia as compared to high fat diet group. It can be concluded that ATC and EZT encapsulated in FODFs provide instant drug release and better therapeutic outcomes.

Keyword: Co-loaded oral films, ezetimibe, atorvastatin, HPMC E5, *In-vitro* drug release, pharmacodynamics.

INTRODUCTION

In Atherosclerotic, cardiovascular patients suffering with ACD (acute coronary disease) are lying at high risk. This life-threatening problem can be curtailed by using statins. To achieve optimum level of LDL-C (<70mg/dL) statins must use with additional lipid lowering agent i.e. by using statins with Ezetimibe 17-23% reduction in LDL-C levels will attain as compared to single statins therapy(Shaya *et al*., 2020). Ezetimibe1-(4-fluorophenyl)-3(R)-[3-(4-fluor ophenyl)- 3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2- azetidinone) belongs to BCS class II, a whitish powder and crystalline in nature, freely soluble in ethanol, methanol, and acetone but insoluble in water, It inhibits biliary and dietary cholesterol absorption in intestinal region (Thompson *et al*., 2016). Atorvastatin Calcium (ATC) is HMG CoA reductase inhibitors or "statins", useful to inhibit conversion of HMG CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol. It reduces Low-density lipoprotein levels (LDL) and triglycerides while increases high-density lipoproteins (HDL)(Laufs *et al*., 2016). ATC is a white crystalline powder, insoluble in acidic aqueous solutions. very slightly soluble in distilled water and freely soluble in organic solvents like methanol and ethanol (Kim *et al*., 2008).

Bioavailability is not disturbed with the administration of statin (HMG-CoA reductase inhibitors), food and antacids. Ezetimibe is a lipid-lowering agent that inhibits almost 54% of cholesterol absorption, which contributes to reduce LDL-C, almost 18-20%, but it does not cause any increase in triglyceride concentration, also reduces the risk of cardiovascular diseases and stroke. The normal dose of Ezetimibe is 10mg/day, but in case of renal impaired patients, dose can increase up to 20mg/day, in close monitoring. Bioavailability of ATC is 12% after oral administration and plasma protein binding is about >98%. Statins are used for the treatment of Hypercholest-erolemia; maintenance dose of Atorvastatin is 10mg/day. Biological half-life of Ezetimibe and ATC 22 and 7 hours, respectively. Due to all of these factors of ATC and EZT an attempt has been done to fabricate instant release oral films to get an increased onset of action, reduce dose frequency and avoid unpredictable results of drug release (Castaneda *et al*., 2017, Chamberlain *et al*., 2018). Their combination empowers additive effect in the inhibition of cholesterol absorption and production. Self- nano emulsifying drug delivery system in the combined therapy

*Corresponding author: e-mail: m.zaman2157@gmail.com

(EZT/ATC), has increased oral bioavailability and improve its ability to better control serum cholesterol levels (Chamberlain *et al*., 2018). In fixed dose combination therapy of ATC and EZT has shown significant reduction in atherogenic lipids that helps in minimization of cardiovascular outcomes (Mostafa *et al*., 2020).

Oral fast dissolving films have better taste masking effect, enhanced dissolution rate and improved bioavailability as compared to market available tablet dosage form (Ma *et al*., 2019). Immediate release buccal films have improved patient compliance by reducing the difficulty of swallowing and by giving immediate effect of drug Immediate oral dissolving film have better effect on patients afraid of vomiting, allergic and coughing attacks, oral films have better taste fast released action, easy administration even without the use of water for swallowing (Zhu *et al*., 2018).

MATERIALS AND METHODS

Materials

Ezetimibe (EZT) and Atorvastatin Calcium (ATC) and HPMC E5 (film former) were gifted by CCL Pharmaceuticals Lahore Pakistan. PEG 400 (Merck, Germany), Tween 80 (Sigma Aldrich, Germany) and Sucrose (Merck, Germany) were used as a plasticizer, solubilizer and sweetener, respectively. Moreover. methanol and ethanol (VWR Shanghai, China) were used as solvents. All the ingredients used were of analytical grade. Locally bred female rats (6 weeks, 150-200g) obtained from animal house of University of Lahore. Equally divided rats of their concerned groups, kept in different cages at animal house (25 + 1^{o}C, RH 45- 50%) for 12hr light/dark alternate cycle by giving them easy access to water and standard diet ad libitum. Fasting about 12-18 hours before starting of experiment and organized according to the protocols given by the Ethics Committee of University of Lahore, Lahore, Pakistan.

Methods

Preparation of ATC/EZT-FDOFs

Solvent evaporation method was adopted for the preparation of films in the current study(Mushtaque *et al*., 2021). Accurately weighed amount of both drugs, EZT in methanol and ATC in ethanol were added separately, to obtain the concentration of 10mg/ml, each of both drugs. The solutions of HPMC E5 (3%), PEG 400 (2%), sucrose (1%) and Tween 80 (2%) were prepared using distilled water as vehicle. Initially, required quantity of plasticizer (PEG-400 solution) was taken in a separate beaker, followed by addition of T80 under continuous stirring for 15 minutes (table 1). EZT and ATC have been added in the said mixture dropwise one after the other, while the mixture was subjected to continuous stirring using hotplate magnetic stirrer. In a separate beaker, the polymer solution was taken and added with sweetener, and mixed for 45 minutes, which was then poured in to the mixture, having drug under continuous stirring, till the homogenous solution has been obtained (Kumar and Yagnesh, 2019). Later on, these mixtures were poured into the glass petri dishes having surface area about 24 cm^2 The petri dishes were placed in a hot air oven at 40 ºC for about 24 hours. After proper drying, each film was peeled off from the petri dish with the help of sharp cutter, wrapped in an aluminum foil to protect it from contamination and kept at room temperature in a desiccator for further use (Ahmad *et al*., 2020, Zaman *et al*., 2019).

Evaluation of Physicochemical and Mechanical Properties of ATC/EZT-FDOFs

Visual inspection was performed to observe the evenness and smoothness of the films surface. Thickness test was performed by the help of digital micrometer (H-2780; Mitutoyo Digital micrometer, USA) (Elagamy *et al*., 2019, Kumar and Yagnesh, 2019) On the other hand, the mechanical strength of the films was judged by folding endurance, in which the film strip was folded for the several times at the same point until it cracks (Zaman *et al*., 2018).Weight uniformity is another essential part in determining the dose of each film formulations, and essential step to find out the drug concentration. Weight calculations can be done by using calibrated electronic weighing balance set to zero before usage. Three films of each formulation were weighed individually and the mean weight was calculated(Savale, 2017). For better patient compliance the compatibility of the film surface pH to that of buccal cavity is very much important, so for the measurement of pH, a strip of 2×2 cm^2 from each film formulation was dissolved in 5ml distilled water and 25CW microprocessor bench top pH/mV meter (BANTE instruments, China) was used to measurement the pH (Irfan *et al*., 2016)

Disintegration time (DT) and total dissolving Time (TDT)

10ml distilled water was taken in a petri dish, previously heated to 37^{o}C. 1×1 cm^2 piece of a strip was placed on the water surface in a petri dish. Strip should float on the surface of water and didn't touch to walls of the petri dish. Stop watch was started while placing the strip, petri dish was slightly shaken. The time when film started to break was noted as DT and when strip was completely dissolved and became the part of the solution was noted as TDT (Patel *et al*., 2016). The DT and TDT of six formulations (F1-F6) were checked to find out best/ optimal formulation.

Drug content uniformity

To find out the drug content uniformity, individual film was dissolved in 100ml phosphate buffer of 6.8 pH and 2ml of this solution was diluted up to 10ml with phosphate buffer. Absorbance was calculated at 232nm and 245nm for EZT and ATC for ATC/EZT-FDOFs

respectively by using a double beam spectrophotometer (8PG instrument T80, UK). Absorbance of samples was compared with absorption of standard dilutions to calculate the percentage drug content.

In-vitro Drug Release

Dissolution apparatus (USP II) was used for *in-vitro* drug release taking phosphate buffer of 6.8 pH as dissolution media under controlled conditions of temperature (37±0.5ºC) and paddle rotation (50 RPM). A Strip of the film having 10mg each of ATC and EZT was taken, fixed on the glass slide with the help of paper clip and placed in the vessel of the dissolution apparatus. The aliquots of 5ml were withdrawn from the vessel after every minute for till the 10^{th} mint, and afterwards at 20^{th} and 30^{th} mints. The equal volume of the dissolution medium was added after every sample with drawn to maintain the constant volume of dissolution medium. The amount of release drug was determined as; (Mady *et al*., 2018, Winarti *et al*., 2021) The *in-vitro* drug release of six formulations (F1-F6) were checked to find out best/optimal formulation.

% Drug Release = (Absorption of the Sample – Absorption of the Standard) × 100

Drug-Excipients Compatibility

FTIR was used to find out any type of interaction between drug and excipients. FTIR spectrum of ATC, EZT, HPMC E5 and the ATC/EZT-FDOFs were taken. The studies were conducted using Agilent Carry 360 (USA).

Scanning Electron Microscope

Surface morphology of FDOFs was studied by using scanning electron microscopy (SEM). It's a technique extensively used to analyze the surface of the desired samples by using electron beam. SEM of EZT-ATC FDOFs was done by placing over the glass slide and then mounted over the stage of microscope to observe the surface morphology at micro level (Aldawsari and Badr-Eldin, 2020).

X-Ray Diffractometry

XRD studies were done to find out any difference in the structure of drugs i.e. crystalline to amorphous form; drug samples were analyzed by X-ray diffractometer. The environment was maintained as, 45 kV tube voltage, 40 mA tube current and scanning angle (2θ) was 5-50°.

Differential scanning calorimetry

Thermo grams of both drugs, (Ezetimibe and Atorvastatin) and film formulations were acquired by using DSC (DSC- 60A thermal analyzer, Shimadzu, Japan).

Thermogravimetric Analysis

Thermo grams of both drugs, (Ezetimibe and Atorvastatin) and film formulations were acquired by using TGA instrument (Pyris diamond series TGA/DTA, USA).

Pharmacodynamics study design

Female albino rats of 6-week age were divided into 4 different groups, each group having 3 rats into it. All animals were fed with specified diet (HC, 2% cholesterol in coconut oil) and general diet for one month except the control group (group 1) that is fed with standard diet and having easy access to clean water *ad libitum*. HF-Diet group (Group 2) (Elagamy *et al.,* 2019). Group 3 and 4 were the test groups treated concomitantly with HC and dugs. Films containing EZT and ATC were given with forceps and standard drugs in the form of aqueous suspension using oral gavage. Treatment was given to 3^{rd} and 4^{th} group after two hours of specified diet. Normal and negative control groups were given by solvent (D.W) for the same experimental period through oral gavage. Preventive phase was completed at the end of 4^{th} week. In curative phase, HF-Diet group (Group 5) was given by standard diet and clean water. Treated groups (group 6 and 7) were just like group 3 and 4 as in preventive phase. Same pattern of dose was given for whole period (one month) and body weight of rats were measured weekly (Abo-shady *et al*., 2020).

All treated groups received ATC/EZT-FDOFs and standard drugs suspension at the dose of 3mg/kg/day. After the completion of experimental time, rats were anesthetized and blood sample was drawn from cardiac region, to perform lipid profile test (HDL, VLDL, LDL, CH, TG) and centrifuged at 4000 rpm for 15 min. The lipid profiles such as cholesterol levels were measured by using a commercial kit ab65390. Total cholesterol, high density lipoproteins (HDL), triglycerides (TG) were calculated and LDL level was determined by using Fried Ewald formula and VLD-L was calculated by the ratio of TG/5 (Hanif and Zaman, 2017). In Histopathological analysis of aorta, first aorta was removed, and its sections were stained with hematoxylin and Eosin (H and E) stains and slides were prepared for the histopathological evaluations(Srivalli and Mishra, 2016).

STATISTICAL ANALYSIS

The results of lipid profiles were evaluated statistically by Analysis of Variances (ANOVA) at 95% confidence interval, using Graph Pad Prism ver.12.

RESULTS

Disintegration Time (DT) and Total Dissolving Time (TDT)

ATC/EZT- FDOFs showed rapid disintegration (11.090±0.4 to 13.09 sec) as well as TDT (2.093±0.03 to 2.456±0.05 min) (table 2). The effect of HPMC E5 and PEG 400 on DT and TDT was observed.

Table 1: Composition for the preparation of ATC/EZT-FDOFs

Formulations	Polymer (mg)	Plasticizer (mg)
F1	150	21.89
F2	150	32.5
F3	125	40
F4	150	43.11
F5	185	32.50
F6	150	21.89
Constant quantities of EZT (10mg) ATC (10mg), solubilizer (%) and sweetener (1%) were used in the formulations		

Table 2: Evaluation parameters ATC/EZT-FDOFs

Formulations	Average weight (mg)	Average Thickness (mm)	Average pH	Moisture loss (%)	Drug contents (%)	DT (sec)	TDT (min)	Folding Endurance
F1	253.633	0.0840	6.02	6.741	108.8	11.477	2.093	302.67
F2	260.567	0.0838	6.07	6.914	105.7	12.602	2.293	304.33
F3	257.867	0.0944	5.68	6.597	101.2	15.113	2.303	314.00
F4	254.100	0.0842	5.96	7.003	101.5	12.107	2.257	329.33
F5	259.967	0.1150	6.47	8.024	97.8	11.090	2.456	305.33
F6	253.900	0.1380	6.02	7.497	99.8	13.090	2.413	315.00

Table 3: Plasma cholesterol and lipid profile in Preventive Studies

Factors (mg/dl)	Normal Group	High Fat Diet Group	ATC/EZT-FODF	ATC/EZT-Suspension
Cholesterol	58.33± 11.59	79.66± 11.54	46.667 ± 2.51	57.33 ± 4.04
HDL	24.667± 8.50	14.33± 5.508	30.66 ± 5.13	26.33 ± 6.80
VLDL	25.33± 6.35	31.00± 3.606	21.33 ± 2.082	22.33 ± 9.29
Triglyceride	107± 14.00	157.66± 31.19	95.66± 19.34	104.66 ± 16.07s

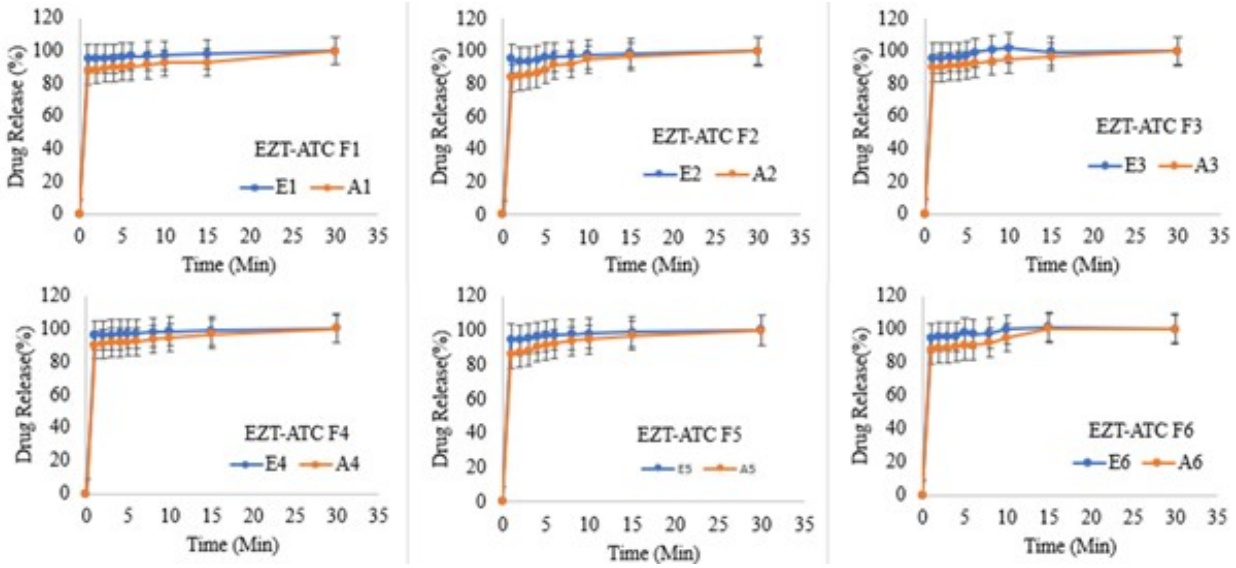

Fig. 1: Illustrating the fast release of ATC and EZT from ATC/EZT-FDOF (F1-F6).

Table 4: Effect of ATC and EZT on Vital Organs of Rats in Preventive Study

Kidneys		Spleen	Liver	Heart		
L	R					
1.12 ± 0.32	1.12± 0.32	0.75±0.01	8.84 ± 1.01	0.93±0.10	Weight (g)	Normal Group
1.33 ± 0.153	1.33 ± 0.15	3.16± 0.11	5.37 ± 0.32	1.25±0.12	Width (cm)	
1.83 ± 0.05	1.83± 0.05	0.99± 0.05	3.90± 0.20	1.25±0.05	Length (cm)	
0.67 ± 0.02	0.67 ± 0.02	0.64± 0.07	5.81 ± 0.49	0.65±0.03	Volume (ml)	
1.47±0.15	1.47±0.15	0.94±0.02	8.93 ±1.04	1.40±0.10	Weight (g)	HFD group
1.41± 0.03	1.41 ± 0.03	3.97±0.15	5.86±0.15	1.50±0.10	Width (cm)	
2.30± 0.10	2.30± 0.10	1.15±0.10	3.92±0.15	1.79±0.10	Length (cm)	
0.85± 0.03	0.85± 0.03	0.83±0.13	6.74±0.47	0.89±0.05	Volume (ml)	
0.49± 0.01	0.49± 0.01	0.36±0.05	4.58±0.61	0.57±0.08	Weight (g)	ATC/EZT-FODF
0.87± 0.06	0.87± 0.06	0.56±0.10	3.83±0.31	0.58±0.02	Width (cm)	
0.79± 0.10	0.79 ± 0.10	0.62±0.04	3.20±0.10	0.59±0.12	Length (cm)	
0.42 ± 0.01	0.42± 0.01	0.43±0.01	3.41±0.73	0.47±0.02	Volume (ml)	
0.53± 0.02	0.53 ± 0.02	0.48±0.03	4.55±0.53	0.63±0.05	Weight (g)	ATC/EZT-Suspension
0.92± 0.05	0.92± 0.05	2.45±0.25	4.00±0.17	0.47±0.08	Width (cm)	
1.39 ± 0.10	1.39± 0.10	0.82±0.05	3.03±0.05	0.69±0.02	Length (cm)	
0.46± 0.03	0.46 ± 0.03	0.23±0.01	3.26±0.10	0.46±0.05	Volume (ml)	

Table 5: Plasma cholesterol and lipid profile in Curative Study

	Normal Group	HF-Diet	ATC/EZT-FDOF	ATC/EZT- Suspension
Cholesterol	56.33± 8.66	77.667 ± 5.77	43.66 ± 7.572	55.33± 4.61
HDL	43.33± 8.08	21.66± 3.215	44.00 ± 3.46	36.00 ± 3.60
VLDL	24.33± 1.15	32.66±6.028	11.66± 1.732	12.66 ± 1.00
Triglyceride	108.66±5.77	139 ± 30.199	88.66 ± 9.86	98.00 ± 6.55

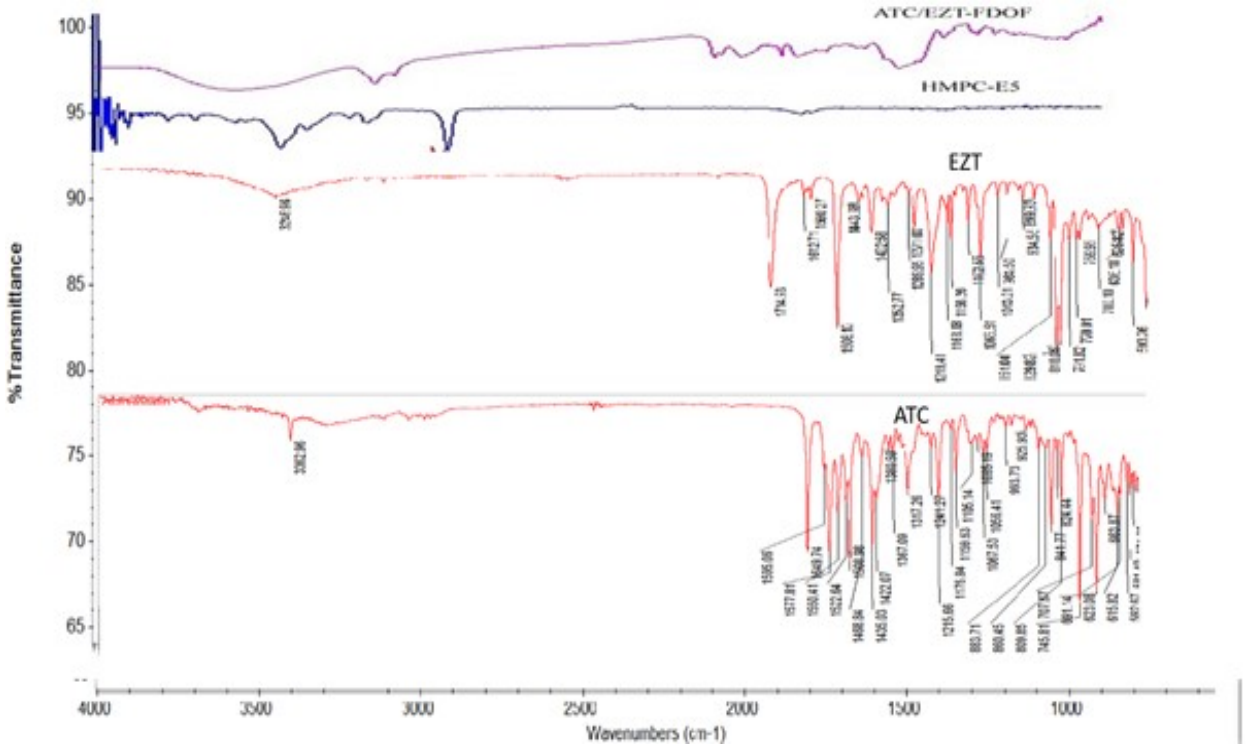

Fig. 2: FTIR spectrum of ATC, EZT, HPMC E5 and ATC/EZT-FDOFs, indicting the chemical compatibilities of the ingredients.

An increase in the time required to break or dissolve was found to be closely associated with the polymeric concentration, as it has direct and positive influence. On the other hand, a negative impact of increase in the concentration of PEG has been noticed, as increase in its concentration showed a decrease in both DT and TDT of the films. Furthermore, the thickness variations of the films were In prepared films, the formulation, weight variation was negligible, which was clearly noticed from the S.D. Uniformity in the weight is an important indicator for the suitability of the process and methodology, adopted for film development. Both PEG 400 and HPMC E5 were found to be slight acidic to basic in nature having pH 5-7 and 5-8 respectively. Such pH range has assisted in preparing a film formulation, compatible with that of oral cavity (5.5-7.4). Though, it was observed that pH found to be increased slightly, with increasing concentrations of plasticizer (PEG 400) and HPME5 (polymer). The added drugs were of acidic nature and at their constant concentration, increased in concentration of the polymer and plasticizer might increase the pH level of ultimate formulations.

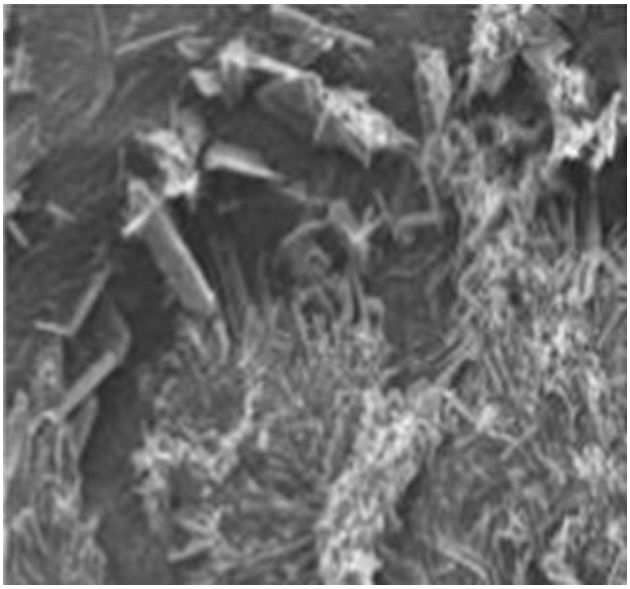

Fig. 3: SEM of EZT/ATC FDOF.

In- vitro Drug Release

The plasticizer to polymer ratio of 4.6:1 found suitable for providing rapid release of both drugs i.e., EZT and ATC. ATC/EZT-FDOFs allowed 87.79% and 97.19% of ATC and EZT to get released in 1 min respectively.

Kinetic models of in-vitro of dissolution data

In-vitro drug release ATC and EZT was fitted in different kinetic models to observe their release behavior and mechanism. EZT exhibited lowest R^2 value for zero order kinetic, directed to that, the drug release per unit time was not constant. The data showed that the best fit model was first order kinetic having the value 0.9823. The value of n from korsmeyer peppas model was 0.1016, indicating fickian type drug diffusion form the films (Srivalli and Mishra, 2016, Sharma and Mehta, 2019). Similar type of behavior was exhibited by the ATC with concentration dependent mechanism of drug release (First order kinetics) followed by ficks law of diffusion having value of n, 0.061 from korsmeyer peppas model (Biplav *et al*., 2018).

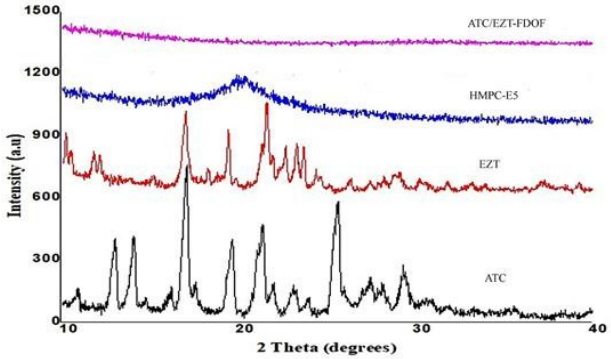

Fig. 4: XRD results of ATC, EZT, HPMC E5 and ATC/EZT-FDOFs, describing the conversion of crystalline form of the drug into amorphous

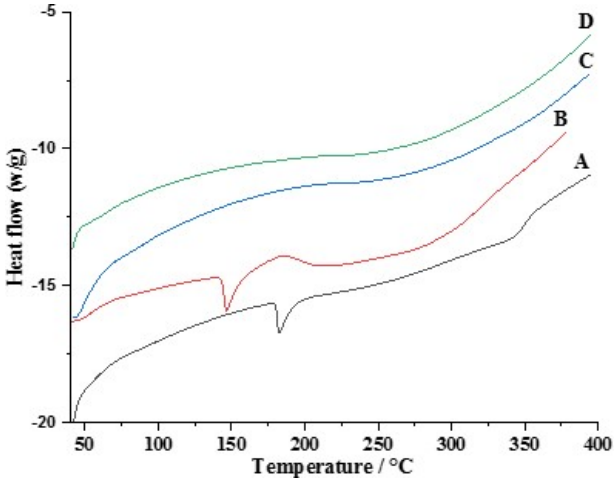

Fig. 5: DSC thermograms of the, (ATC) A, (EZT) B, (HMPC-E5) C and (ATC/EZT FODF) D.

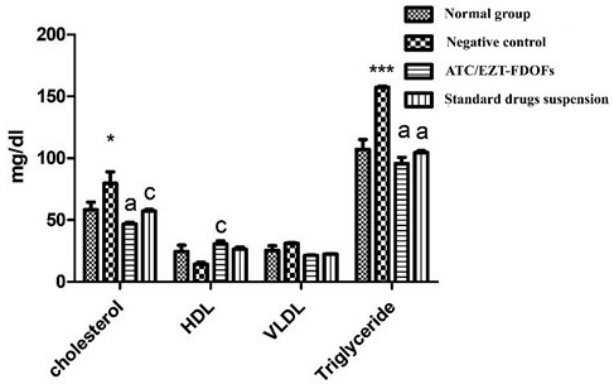

Fig. 6: Describing the effects on cholesterol, HDL, VLDL and Triglycerides during preventive treatment in Normal group, HF-Diet control, treated with ATC/EZT-FDOFs and Standard drugs suspension.

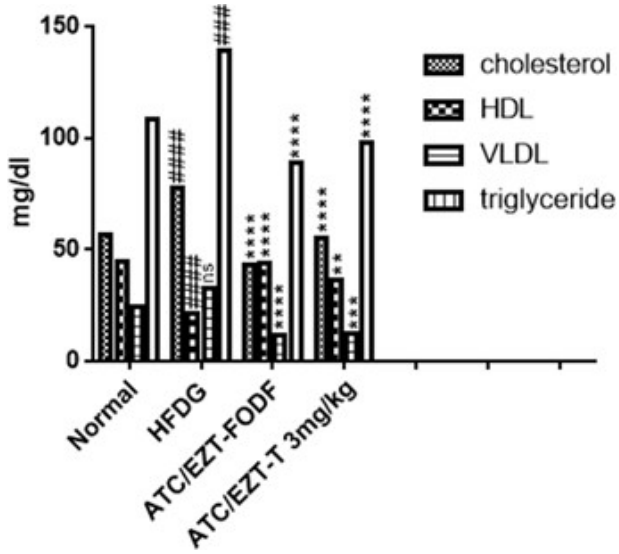

Fig. 7: Describing the effects on cholesterol, HDL, VLDL and Triglycerides during curative treatment in Normal group, Ne HF-Diet gative control, ATC/EZT-FDOFs treated and treated with Standard drugs suspension

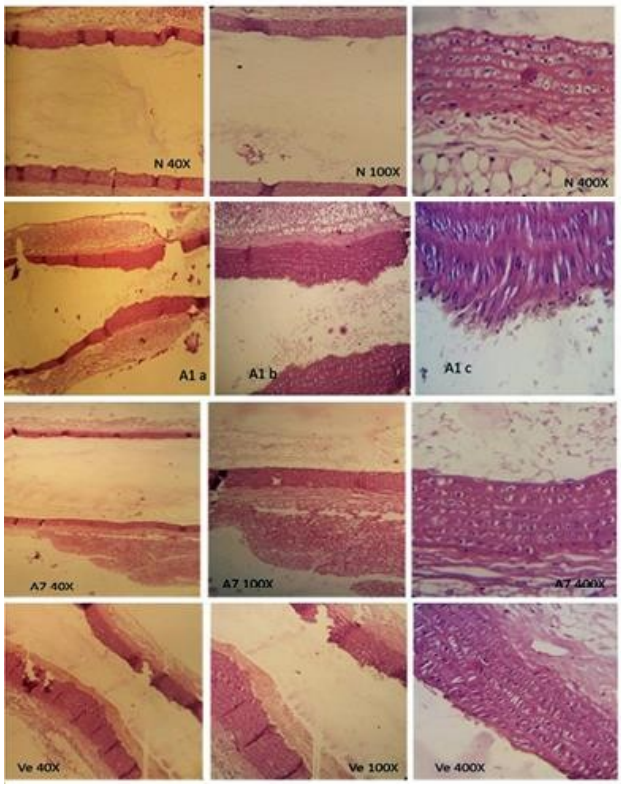

Fig. 8: Histopathological evaluation of Aorta, describing comparative analysis of Control (N), HF-Diet (A1), ATC/EZT-FDOFs treated (A7) and treated with Standard drug suspension (Ve).

Chemical compatibility of the ingredients

FTIR spectrum of ATC showed peak at 1775 cm^{-1} by indicating the presence of C=O stretching peak of carboxylic acid. Peak present at 1125cm^{-1} showed C-F stretching and at 3271cm^{-1} O-H stretching and peak at 3354cm^{-1} were showed the stretching due to N-H, aliphatic primary amine group. FTIR spectrum of EZT showed wide band at 1165 cm^{-1} by assuring the presence fluorine group by stretching of C-F. Peak present at 1755cm^{-1} C=O stretching indicating the presence of carboxylic acid, peak at 3214 cm^{-1} showed the stretching due to O-H, alcoholic group. peak at 3474 cm^{-1} showed the stretching due to N-H, amine group. In the spectrum of HPMC E5, a peak showed at 1272cm^{-1}, that confirms the presence of -C-O stretching peak of aromatic ester group. Peak at 1750 cm^{-1} showed the presence of C-H stretching, which indicated aromatic compounds (fig. 2). FTIR spectrum of ATC/EZT-FDOFs showed peak at 3260cm^{-1} O-H stretching and peak at 3504cm^{-1} were showed the stretching due to N-H, aliphatic primary amine group (Arunkumar *et al*., 2019).

Scanning electron microscope

SEM has given detailed high-resolution images of sample surface by adjusting electron beam microscope. The surface morphology of formulated FDOFs of ATC-EZT was presented in fig. 3. SEM revealed that ATC-EZT FDOF has little bit crystalline nature while FDOF showed that the EZT, ATC and polymer were mixed thoroughly and created a mesh like appearance.

X-Ray diffractometry

XRD was performed to find out the nature of drugs, either it is crystalline or amorphous. XRD pattern of ATC intense peaks at diffraction angle (2θ) of 13.007°, 14.04°, 17.03°, counts were 442, 450, 798, 438, 509, 623 and 314. XRD pattern of EZT reviled intense peaks at diffraction angle (2θ) of 10.2°, 11.78°, 16.9°, 19.36°, 21.54°, 22.63°and 23.24° and their corresponding counts were 323, 233, 427, 340, 471, 262 and 272.

Similarly, HPMC E5 showed low intensity peaks at diffraction angels (2θ) of 10.12°, 11.01°, 13.739°, 16.25°, 18.73°, 19.83° and 25.60°and their corresponding counts were 233, 235, 191, 182, 219, 295 and 13119.59°, 21.339°, 25.6° and 29.28° and their corresponding (fig. 4). ATC/EZT-FDOFs diffractogram exhibited that the sharpness of the peaks has been diminished. It was the clue of that, both drugs have lost their crystallinity and had been converted into amorphous form. Which might be the reason better dissolution and release of the drug from the films.

Differential Scanning Calorimetry

In DSC analysis both drugs Atorvastatin and Ezetimibe exhibit endothermic peaks at 182^{o}C and 146^{o}C respectively (Zaman *et al*., 2016). These values correspond to the melting points of the pure drugs as reported in literature, that indicates the purity of drug (Rani, 2016). Formulation F2 (fig. 5) doesn't show any endothermic peak and indicates minor behavior, that

Table 6: Effect of ATC and EZT on Vital Organs of Rats in Curative Study

Kidneys		Spleen	Liver	Heart		
L	R					
1.15± 0.09	1.15± 0.09	0.69±0.10	8.645±1.01	0.93±0.10	Weight (g)	Normal Group
1.20± 0.15	1.20± 0.15	1.06±0.11	5.34± 0.32	1.05±0.10	Width (cm)	
1.85± 0.05	1.85± 0.05	0.93±0.02	3.75 ± 0.20	1.20±0.17	L (cm)	
0.56± 0.02	0.56± 0.02	0.66±0.04	5.50± 0.52	0.74±0.03	Vol (ml)	
1.03± 0.02	1.03± 0.02	0.51±0.02	7.35± 0.46	0.81±0.08	Weight (g)	HF-Diet
1.15 ± 0.15	1.15±0.15	2.45±0.02	3.52±0.28	1.02±0.05	Width (cm)	
1.15± 0.05	1.15± 0.05	0.90±0.10	3.25±0.10	1.10±0.10	L (cm)	
0.62± 0.05	0.62±0.05	0.81±0.13	6.25±0.24	0.75±0.05	Vol (ml)	
0.57± 0.02	0.57±0.02	0.42±0.02	4.41± 0.38	0.52±0.03	Weight (g)	ATC/EZT-FODF
0.80± 0.06	0.80± 0.06	0.72±0.10	2.50± 0.10	0.32±0.02	Width (cm)	
0.763±0.05	0.76±0.055	0.570±0.07	2.06± 0.05	0.42±0.02	L (cm)	
0.37±0.02	0.37± 0.02	0.33±0.017	3.09± 0.09	0.42±0.02	Vol (ml)	
0.65± 0.05	0.65± 0.05	0.43± 0.02	4.78± 0.28	0.39±0.00	Weight (g)	ATC/EZT-Suspension
0.90± 0.05	0.90 ± 0.05	1.95± 0.05	2.78 ± 0.10	0.55±0.05	Width (cm)	
0.92± 0.02	0.92± 0.02	0.65± 0.12	2.16±0.05	0.39±0.02	L (cm)	
0.57± 0.03	0.57± 0.03	0.31± 0.01	3.69± 0.27	0.40±0.01	Vol (ml)	

means polymer drugs compatibility. DSC thermogram of both drugs with polymer shows that there is no interaction between both drugs and polymer.

Pharmacodynamics study

Pharmacodynamics studies have been distributed in two phases, first one was preventive model and the second phase, curative model.

Drug treatment and its effects in Preventive Study

In preventive treatment group, the combination of ATC/EZT suspension and FODF formulation were administered and compared with HF-Diet group. The results have shown significantly low hyperlipidemic profile in the groups, which were receiving the preventive treatment with high fat diet in comparison to the other group, with no preventive treatment. Similarly, plasma VLDL and triglycerides level of both groups receiving suspension and FODF preventive treatment are very low as compared to HFD group and FODF receiving group have significantly lower level of above two parameters as compared to suspension receiving group even lower than the normal group (table 3).

Lipid profile in normal Vs HFD group

Cholesterol level, HDL and VLDL showed a non-significant increase when compared with HF-Diet group.

Lipid profile in HFD group vs treated groups

HFD group demonstrated a significantly increased levels of cholesterol and triglyceride as compared to the normal group. When ATC/EZT-FODF are compared with HFD group, it showed highly significant increase in cholesterol and triglycerides while in HDL level, the difference was significant effect. When ATC/EZT standards were compared with HF-Diet group, it showed highly significant increase in triglycerides and a significant increase in cholesterol. (a stand for highly significant and c stands for significant) (fig. 6). The ATC/EZT-FODF showed even better results than that that of standard. The one reason might be the better dissolution due to improved solubility.

Whereas a highly significant ($p<0.001$) increase in triglyceride level.

of the drug. During films preparation, the drugs were first converted into solution form, allowing to attain the smallest possible size of the drug particles. Moreover, the addition of PEG 400 and tween 80 also assisted the solubilization of the poorly water-soluble drugs. This processing has converted the crystalline drugs into amorphous form, that could be observed by the XRD graphs. Amorphous form of the drugs and reduced particle sizes of the drugs not only allowed rapid drug release and better absorption of the drug, but also the greater surface area might have provided better opportunities for the drugs to bind with receptors more firmly. It might lead to the better therapeutic response.

Effect on Rat's vital organs in curative phase

After dissection of all rats in preventive study, weight of different organs of animals were weighed and compared with HF-Diet. Effect of treatment was significant on all vitals organ's weight, width, length, and volume, compared to HFD group (table 6). Histopathological evaluation.

Aorta was selected from all groups. Wash it with normal saline and then preserved in 10% Formaline solution for histopathological investigation. Tissus of Aorta was stained with hematoxylin and eosin for its evealuation

(fig. 8). Images of Aorta from all groups were taken at different Magnification powers (40 40x, 100x and 400x).

Effect on Rat's vital organs

After dissection of all rats in preventive study, weight of different organs of animals were weighed and compared with HF-Diet group. Effect of treatment was significant on all vitals organ's weight, width, length, and volume, compared to n HF-Diet group. The weight and size of heart is reduced receiving preventive treatment and rats of HF- Diet group. The vital organs weight, width, length and volume were found less than both normal and HFD group (table 4) having description about these results.

Drug treatment and its effects in Curative Study

In curative model, cholesterol level of selected rats was already high, just treatment was given to rats for 4 weeks. Body weight of all rats was weekly checked and after the completion of study necropsy has been done. The values of plasma lipoprotein were observed and histopathology of aorta has been done.

Plasma Lipoprotein Analysis in Curative Study

Dissection of rats has been done after 8-10 hours of fast. Blood sample was collected to observe HDL, VLDL, total plasma cholesterol and triglycerides by using commercial enzymatic assays (table 5).

Lipid profile in normal Vs HF-Diet group

Cholesterol level showed a significant effect ($p<0.05$) when compared normal group with HF-Diet group. While triglycerides showed a highly significant ($P<0.001$) effect. HDL and VLDL showed non-significant results.

Lipid profile in normal Vs HF-Diet group in Curative Study

Cholesterol, HDL and triglycerides are highly significant in HFD group when compared with normal. Cholesterol level, HDL, VLDL and triglycerides, when compared with HFD group, they were found to be highly significant ($p<0.001$) in ATC/EZT-FODF. While standard drug showed highly significant effect on cholesterol, VLDL and triglycerides, while significant in HDL when compared with HF-Diet group. (*** indicates highly significant and ** for significant) (fig. 7)

DISCUSSIONS

Disintegration Time (DT) and Total Dissolving Time (TDT)

Usually, it was noticed that an increase in concentration of PEG 400 (plasticizer) would be the cause of decrease in the dissolution time and an increase in polymer concentration may delay it This might be accredited by the fact that the resistance against the solubility of HPMC, was due to the presence of PEG 400 that might be seeped out from the strips in the dissolution medium. Due to loss of PEG 400 from the films, penetration of the water substance increases, thus it lowered down the DT and TDT of the films. The obtained results had clearly indicated the objective of the studies i.e., to formulate the film formulation, capable of delivering the drug promptly, was satisfied. Similarly, greater plasticizer concentration might increase the FE values, as it increases the strength of the film. The surface morphological evaluation by visual inspection has exhibited apparently smooth surface of the films having a uniform distribution of drug and other excipients, as there were no any individual particle of drug or other excipients to be identified.\It seemed that drug is present in the form of solid-solid solution with the excipients. Uniform, distribution is one of the key factors in formulation preparation, as it confirms the dose uniformity and drug contents.

Folding endurance is an imperative parameter to judge the mechanical strength of the prepared films. The observation has been made that polymer and plasticizer have opposite impact from each other, on mechanical properties of the films. Polymer found to be decreasing the mechanical strength, while plasticizer has the ability to enhance it. Same observations existed in the current studies, where formulations with greater PEG contents were more mechanically strong. Prepared formulations of ATC/EZT-FDOFs were found to have uniform thickness with in the same formulation.

The results were indicating, that the thickness and weight, both were directly related to the amount of ingredients in each formulation directly influenced by the variation in the weight of the films(Khodaei *et al*., 2020).

In vitro drug release

Drug release studies were of primary importance in the development of a drug delivery system, and for optimization of excipients is the basic phenomenon. For the development of a suitable film formulation, the concentrations of both polymer and plasticizer play the critical role. Their ratios are deciding factor for the rate of dissolution, DT, TDT, mechanical strength and the rate of release of drug. The findings have also showed that tween 80 might has assisted both the hydrophobic drug to get solubilize and dissolve more instantly (fig. 1).

Histopathological findings of Aorta

Normal N (40X, 100X and 400X) Endothelial lining indicates uniform intact layer, uniform thickness of tunica media and adventia is also visible. So there is No sign of atherosclerosis and necrosis has been observed.

A1 (40X, 100X and 400X) Localized enlargement of the tunica media and intima. Most of the endothelial lining was intact however more than half of the endothelial layer harbored brown pigment containing cells which may lead to atherosclerosis. A7 (40X, 100X and 400X) Intact endothelial lining with uniform tunica media and

adventia. No sign of atherosclerosis or necrosis has been reported here.

Ve (40X, 100X and 400X) Endothelial cell layer is normal. Uniform thickness of tunica media and tunica adventia has been seen. Localize disarrangement of the elastic tissue was noticed. Overall, no sign of necrosis or atherosclerosis. The endothelial cells have basophilic pigment pink cytoplasm. Occasionally endothelial cells contained brownish.

CONCLUSION

The current study was adapted to prepare and evaluate the fast-dissolving oral films of already approved combination therapy of Atorvastatin and Ezetimibe in treatment of hyperlipidemia. The prepared formulation has effective and satisfactory physiochemical and therapeutic effect. The combination of both drug in single film may helpful to increase the patient compliance by reduction in dose frequency and failure to take dose as well as rapid onset of action and also mitigate the cost of treatment. There is further need to study the *in vivo* pharmacokinetic studies to reveals the better outcomes of FODFs in hyperlipidemia patients.

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