RESEARCHE ARTICLE

Comparative Hypoglycaemic Activity of Fixed Dose Combination Anti-Diabetic Formulations Versus Respective Monotherapies in Diabetic Rabbits

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ABSTRACT

Type 2 diabetes mellitus is a disease of impaired glucose homeostasis and chronic hyperglycemia. Current approaches for the treatment frequently involving the use of combination therapy. The aim of the present study was to evaluate and compare the hypoglycaemic activity of fixed-dose combination anti-diabetic formulations and respective individual agents using rabbits as an animal model. Experimental diabetes was induced by a single intravenous injection of alloxan monohydrate at a dose of 150 mg/kg. Individual drugs and combination tablets were administered to experimental groups. Fasting blood glucose level was estimated at 0, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, and 72 hours using glucometer. Data were statistically analyzed using student t-test and $p < 0.05$ considered as statistically significant. The reduction in fasting blood glucose level in diabetes-induced rabbits was significantly higher with combination products compared to individual drugs. Fixed-dose combination products had shown improved glycaemic control than individual agents. Fixed-dose combination therapy can be used as a suitable option for selected patients requiring multiple glucose-lowering therapies for use as an adjunct to diet and exercise to improve glycaemic control in type 2 diabetes mellitus.

Keywords: Empagliflozin, Linagliptin, Metformin hydrochloride, Fixed-dose combination, Animal models, Hypoglycaemic activity.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disease that is heterogeneous in both pathogenesis and clinical manifestation, there is an unmet need for new anti-diabetic therapies and concomitant treatment options for T2DM in patients who are not adequately controlled on monotherapy. Current treatment approaches involving a stepwise approach to the use of combination therapy with two or more anti-diabetic agents with complementary modes of action are frequently required. Empagliflozin is a Sodium-glucose co-transporter 2 (SGLT2) inhibitor, Linagliptin is a dipeptidyl peptidase 4 (DPP-4) inhibitor and Metformin is a biguanide approved for use as an adjunct to diet and exercise to improve glycaemic control in adults with T2DM. Combination therapy with these two or more drugs has the potential to offer improved glycaemic control compared with that achieved with the individual agents, leading to a simplified dosing regimen, which may assist in adherence to therapy.[1-3] Comparison between Fixed-dose combination products and monotherapy is presented in Figure 1.

Formulation and process optimization of empagliflozin and linagliptin immediate release (IR) tablets, empagliflozin, and metformin hydrochloride extended-release (ER) tablets and empagliflozin, linagliptin and metformin hydrochloride extended-release (ER) tablets were covered in our earlier investigations. The present study was designed to evaluate and

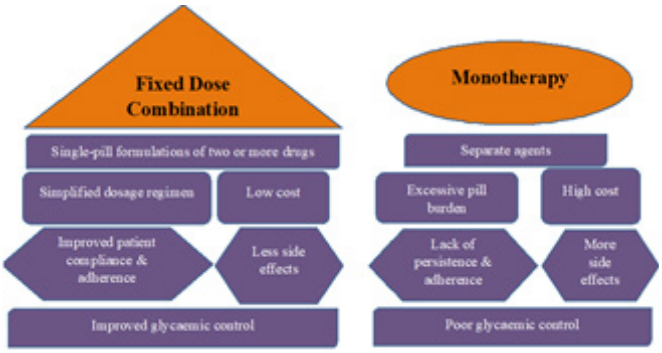

Figure 1: Comparison between fixed-dose combination products and monotherapy.

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compare the hypoglycaemic activity of fixed dose combination products and respective individual drugs in diabetes-induced rabbits as animal model.

MATERIALS AND METHODS

Materials

Empagliflozin, Linagliptin and metformin hydrochloride were received as a gift sample from Aurobindo Pharma Ltd., Hyderabad. Sodium carboxymethyl cellulose 7HF PH (Ashland), HPMC K 100M CR (Dow chemicals), Microcrystalline cellulose Avicel PH112 (FMC), Mannitol Pearlitol SD 200 (Roquette), Hydroxypropyl cellulose Klucel LF and SSL (Nippon soda), Colloidal silicon dioxide (Evonik), Magnesium Stearate (Peter greven), Lactose spray-dried Supertab 11SD (DFE), Croscarmellose sodium AC-DI-SOL (FMC), Meglumine (Merck), Crospovidone Polyplasdone XL 10 (Ashland) and Iron oxide yellow (Neelikon) were used as received. Alloxan was procured from Sigma Chemical Co.

Methods

Preparation of fixed-dose combination (FDC) formulations

Preparation of empagliflozin and linagliptin IR tablets

Hydroxypropyl cellulose was dissolved in purified water under stirring till a clear solution was formed and linagliptin was dissolved in it and stirred for another 30 min. Intragranular materials mannitol and Microcrystalline cellulose were sifted together through ASTM mesh #40, loaded into a fluid bed processor (FBP, top spray), and prewarmed with minimum effective fluidization for 10 min at inlet temperature of 35–40°C. Binder solution was sprayed onto intragranular material in FBP at a product temperature of 27 to 30°C. After spraying, wet mass was dried for 30 min at product temperature of 35°C to 45°C and checked LOD at 105°C (Limit: NMT 2.5% w/w). Dried granules were sifted through ASTM mesh #30 and retain were milled using #40G screen in co-mill. Extra granular materials (Empagliflozin, microcrystalline cellulose, crospovidone, and aerosil) were cosifted with milled granules through ASTM mesh #30 and mixed in a low shear double cone blender for 20 minutes. Then lubricated with magnesium stearate (sifted through ASTM mesh #40) for 5 min in the blender. Lubricated blend was compressed into tablets. The optimized formulation composition of empagliflozin and linagliptin IR tablets is presented in Table 1.

Table 1: Optimized formulation composition of empagliflozin and linagliptin IR tablets

S. No.	*Ingredients*	*Quantity (mg/tablet)*
I	Intragranular	
1	Mannitol (Pearlitol SD 200)	90.00
2	Microcrystalline cellulose (Avicel PH 112)	15.00
II	Binder solution	
3	Hydroxypropyl cellulose (SSL)	15.40
4	Linagliptin	5.00
5	Purified water	Q.S.
III	Extragranular	
6	Empagliflozin	25.00
7	Microcrystalline cellulose (Avicel PH 112)	32.40
8	Crospovidone (Polyplasdone XL 10)	33.00
9	Colloidal silicon dioxide (Aerosil 200 pharma)	2.00
10	Magnesium Stearate	2.20
Total tablet weight		220.00

Preparation of empagliflozin and metformin hydrochloride ER tablets (Bi layer tablets)[4]

IR part blend of empagliflozin and ER part blend of metformin hydrochloride were prepared individually. Metformin hydrochloride part granules were prepared using wet granulation technology (Rapid mixer granulator, make: Gansons). Dry mix material (metformin hydrochloride and sodium CMC) were granulated using purified water as a granulating fluid in RMG at impeller slow speed and chopper slow speed. Wet mass was dried in a rapid dryer for 45 -60 min at the inlet temperature of 50 ± 5°C. Dried granules (LOD at 105°C: 0.5 to 1.5%w/w) were passed through ASTM mesh #20 and retains were milled using co-mill fitted with screen 1016 micron at slow to medium speed. Extra granular materials (ER polymer and Aerosil) and milled granules were co-sifted through ASTM mesh #20 and blended in a low shear double cone blender for 10 minutes. Then lubricated with magnesium stearate for 5 minutes in the blender. Empagliflozin part blend was prepared by direct blending approach. empagliflozin, lactose spray dried, microcrystalline cellulose, hydroxypropyl cellulose, croscarmellose sodium, colloidal silicon dioxide and iron oxide yellow were sifted together through ASTM mesh #30 and mixed in a low shear double cone blender for 20 minutes. Then lubricated with magnesium stearate for 5 minutes in the blender. Both empagliflozin part and metformin hydrochloride part blends were compressed into bilayer tablets using Eliza Press. Optimized formulation composition of empagliflozin and metformin hydrochloride ER tablets is presented in Table 2.

Preparation of empagliflozin, Linagliptin and metformin hydrochloride ER tablets (Bi layer tablets)

IR part blend of empagliflozin, linagliptin, and ER part blend of metformin hydrochloride were prepared separately. Metformin hydrochloride part granules were prepared as described in empagliflozin and metformin hydrochloride ER tablets preparation procedure. Empagliflozin and linagliptin part blend was prepared by using fluid bed processor. Hydroxypropyl cellulose and meglumine were dissolved in purified water under stirring till clear solution was formed and linagliptin was dispersed in it and stirred for another 30 minutes. Granulation and blending process is followed as described in empagliflozin and linagliptin IR tablets preparation procedure. Both empagliflozin, linagliptin part and metformin hydrochloride part blends were compressed into bi-layer tablets using Eliza Press. Optimized formulation composition of empagliflozin, linagliptin and metformin hydrochloride ER tablets is presented in Table 3.

Table 2: Optimized formulation composition of empagliflozin and metformin hydrochloride ER tablets

S. No.	*Ingredients*	*Quantity (mg/tablet)*
I	Empagliflozin part (IR part), Layer 1	
	Pre-lubrication	
1	Empagliflozin	25.00
2	Lactose spray dried (Supertab 11SD)	59.00
3	Microcrystalline cellulose (Avicel PH112)	90.00
4	Hydroxypropyl cellulose (Klucel LF)	19.80
5	Croscarmellose sodium (Ac-Di-Sol)	22.00
6	Colloidal silicon dioxide (Aerosil 200 pharma)	2.00
7	Iron oxide yellow	0.20
	Lubrication	
8	Magnesium Stearate	2.00
	Empagliflozin part weight	220.00
II	Metformin hydrochloride part (ER part), Layer 2	
	Intragranular	
9	Metformin hydrochloride	1000.00
10	Sodium CMC (7HF PH)	65.00
	Binder	
11	Purified water	Q.S.
	Extra granular	
12	HPMC K 100M CR	221.00
13	Colloidal silicon dioxide (Aerosil 200 pharma)	7.50
14	Magnesium Stearate	6.50
	Metformin hydrochloride part weight	1300.00
	Total tablet weight (Bi-layer)	1520.00

Table 3: Optimized formulation composition of empagliflozin, linagliptin and metformin hydrochloride ER tablets

S. No.	*Ingredients*	*Quantity (mg/tablet)*
I	Empagliflozin, Linagliptin part (IR part), Layer 1	
	Intragranular	
1	Mannitol (Pearlitol SD 200)	78.00
2	Microcrystalline cellulose (Avicel PH 112)	15.00
	Binder solution	
3	Hydroxypropyl cellulose (SSL)	15.40
4	Meglumine	12.00
5	Linagliptin	5.00
6	Purified water	Q.S.
	Extragranular	
7	Empagliflozin	25.00
8	Microcrystalline cellulose (Avicel PH 112)	32.40
9	Crospovidone (Polyplasdone XL 10)	33.00
10	Colloidal silicon dioxide (Aerosil 200 pharma)	2.00
11	Iron oxide yellow	0.20
12	Magnesium Stearate	2.00
	Empagliflozin and Linagliptin part	220.00
II	Metformin hydrochloride part (ER part), Layer 2	
	Intragranular	
13	Metformin hydrochloride	1000.00
14	Sodium CMC (7HF PH)	65.00
	Binder	
15	Purified water	Q.S.
	Extra granular	
16	HPMC K 100M CR	221.00
17	Colloidal silicon dioxide (Aerosil 200 pharma)	7.50
18	Magnesium Stearate	6.50
	Metformin hydrochloride part weight	1300.00
	Total tablet weight (Bi-layer)	1520.00

In vitro Studies

Assay

Assay of empagliflozin and linagliptin IR tablets, empagliflozin and metformin hydrochloride ER tablets, and empagliflozin, linagliptin and metformin hydrochloride ER tablets performed using the HPLC method.[5-6]

Dissolution

The dissolution test was performed using USP Type-I (Basket), with a volume of 900 mL, at a stirring speed of 100 rpm and the temperature was maintained at 37°C ± 0.5°C. The study was carried out in pH 6.8 phosphate buffer separately for individual components for all combination products at 1, 2, 4, 6, 8, 10, and 12 h for metformin hydrochloride part and 10, 15, 20, 30, 45 and 60 min for empagliflozin and or Linagliptin part respectively.[7]

Drug Release Kinetics

The optimized formulation of empagliflozin and metformin hydrochloride ER tablets and empagliflozin, linagliptin and metformin hydrochloride ER tablets were evaluated using various mathematical equations.[8]

Stability Studies

Stability studies were conducted on the optimized formulation of empagliflozin and linagliptin IR tablets, empagliflozin and metformin hydrochloride ER tablets, and empagliflozin, linagliptin, and metformin hydrochloride ER tablets as per international conference of harmonization (ICH) guidelines at an accelerated (40°C/75% RH) and long term (25°C/60% RH) stability conditions.[9]

Evaluation of Hypoglycaemic Activity

Study design

Adult healthy albino rabbits (2.5-3.0 kg) were taken for the study and divided into eight groups, each consisting of six animals. Animals were housed under the standard conditions of temperature (22-25°C), humidity (55 ± 15%) and light 12 hrs/day for 7 days. Group-A served as untreated normal control. Animals were fasted overnight and divided into seven groups of six animals each. Diabetes was induced with a single intravenous injection of 150mg/kg bodyweight of alloxan monohydrate dissolved in isotonic saline and animals are fed

with 5% glucose solution to avoid drug-induced hypoglycemia. Group-B is considered as diabetic control. The blood glucose levels of animals were measured after 48 hours of alloxan injection through ear vein tipping using a glucometer (Accu Chek Active blood glucose meter kit). Rabbits showing blood glucose levels above 200 mg/dl were considered diabetic and included in the study.

Dosage of empagliflozin, linagliptin, and metformin hydrochloride is 25, 5 m, and 1000 mg respectively in humans (70 kg body weight). Animal dose calculated for empagliflozin, linagliptin and metformin hydrochloride is 1.10, 0.22, and 44.30 mg respectively. Individual drugs are administered as a solution to group-C, D, and E. Combination products are administered by compressing mini tablets containing the required equivalent amount of animal dose. Mini tablets of empagliflozin and linagliptin IR tablets with an average weight of 9.7 mg (equivalent to 1.10 mg empagliflozin and 0.22 mg linagliptin) were administered to group-F. Mini tablets (bi-layer) of empagliflozin and metformin hydrochloride ER tablets were compressed with 57.6 mg of metformin part blend (equivalent to 44.30 mg metformin) and 9.7 mg of an empagliflozin part blend (equivalent to 1.10 mg empagliflozin) and administered to group-G. Mini tablets (bi-layer) of empagliflozin, linagliptin and metformin hydrochloride ER tablets were compressed with 57.6 mg of metformin part blend (equivalent to 44.30 mg metformin) and 9.7 mg (equivalent to 1.10 mg empagliflozin and 0.22 mg linagliptin) and administered to group-H.[10-12]

The details of experiment groups are as below:

- Group-A: Normal control
- Group-B: Diabetic control
- Group-C: Diabetic + Empagliflozin reference (Empagliflozin API, as solution)
- Group-D: Diabetic + Linagliptin reference (Linagliptin API, as solution)
- Group-E: Diabetic + Metformin reference (Metformin API, as solution)
- Group-F: Diabetic + Empagliflozin and linagliptin IR tablets
- Group-G: Diabetic + Empagliflozin and metformin ER tablets
- Group-H: Diabetic + Empagliflozin, linagliptin and metformin ER tablets

The blood glucose levels of animals were measured at 0, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h through ear vein tipping using a glucometer. After collection of blood, the pricked

Table 4: *In vitro* evaluation of empagliflozin and linagliptin IR tablets

Component	*Empagliflozin*		*Linagliptin*	
Assay (%)	99.7		100.2	
Dissolution	% drug dissolved (Mean ± RSD)			
Time (min)	Marketed product (Empagliflozin IR tablets)	Test (FDC)	Marketed product (Linagliptin IR tablets)	Test (FDC)
0	0	0	0	0
10	65 ± 1.32	72 ± 1.84	61 ± 2.21	66 ± 1.51
15	77 ± 1.02	79 ± 1.42	75 ± 3.21	78 ± 1.05
20	82 ± 1.23	84 ± 0.52	84 ± 1.21	84 ± 0.42
30	90 ± 0.23	88 ± 0.41	88 ± 1.51	87 ± 0.89
45	98 ± 0.52	100 ± 0.74	97 ± 0.57	101 ± 0.42
f2		70	--	75

Table 5: *In vitro* evaluation of empagliflozin and metformin hydrochloride ER tablets

Component	*Empagliflozin*		*Metformin hydrochloride*		
Assay (%)	99.4		99.6		
Dissolution	% drug dissolved (Mean ± RSD)				
Time (min)	Marketed product (Empagliflozin IR tablets)	Test (FDC)	Time (h)	Marketed product (Metformin SR tablets)	Test (FDC)
0	0	0	0	0	0
10	65 ± 1.32	58 ± 2.10	1	26 ± 3.41	21 ± 1.06
15	77 ± 1.02	71 ± 1.52	2	41 ± 2.56	37 ± 1.84
20	82 ± 1.23	78 ± 0.56	4	57 ± 3.41	53 ± 1.07
30	90 ± 0.23	84 ± 1.03	6	65 ± 2.36	61 ± 1.64
45	98 ± 0.52	96 ± 0.56	8	78 ± 1.84	74 ± 1.23
60	99 ± 0.41	98 ± 0.42	10	86 ± 1.75	83 ± 0.96
		63	12	96 ± 0.85	97 ± 0.41
f2					
		f2		70	

site of vein was pressed with a cotton swab soaked in ethanol to protect the rabbit against infection. Statistical analysis of data was carried out by employing student t-test and $p < 0.05$ considered as statistically significant. Percent reduction in fasting blood glucose levels of FDC tablets and individual dugs treated experimental groups is calculated by comparing levels at each sampling time points against pre dose (0 h) interval of that particular group. The study was approved by the Institute Animal Ethical Committee (IAEC) with the registration No.: IAEC.1821/PO/Re/S/15/CPCSEA/345/2017.

RESULTS AND DISCUSSION

In vitro Evaluation

Assay and drug release profile of the optimized formulations of all FDC formulations were evaluated and dissolution profile of combination tablets is compared with individual marketed formulation. *In vitro* evaluation data of all three combination products are presented in Tables 4–6 and comparative dissolution profiles of marketed product and FDC test product are presented in Figures 2–4. A F2 value of all the dissolution

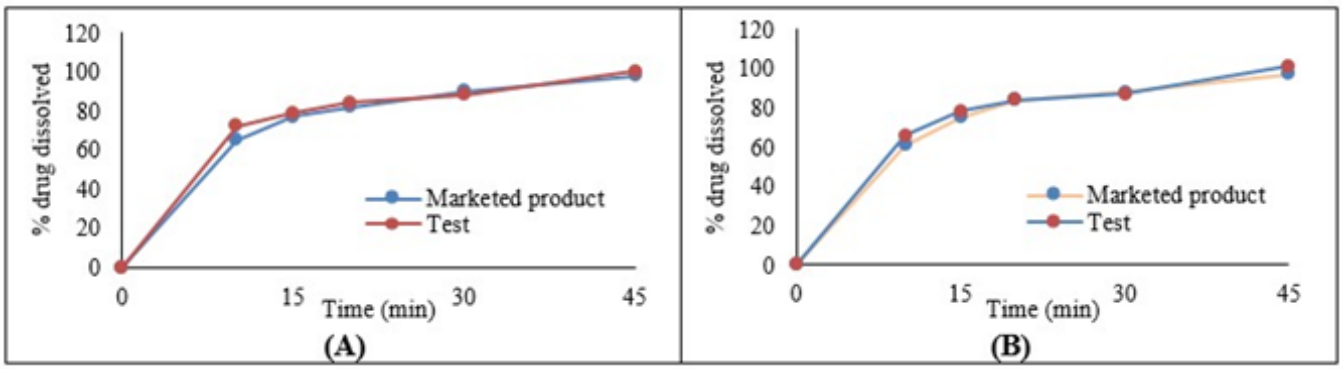

Figure 2: Comparative dissolution profile Empagliflozin and Linagliptin IR tablets.
(A): Empagliflozin part; (B): Linagliptin part.

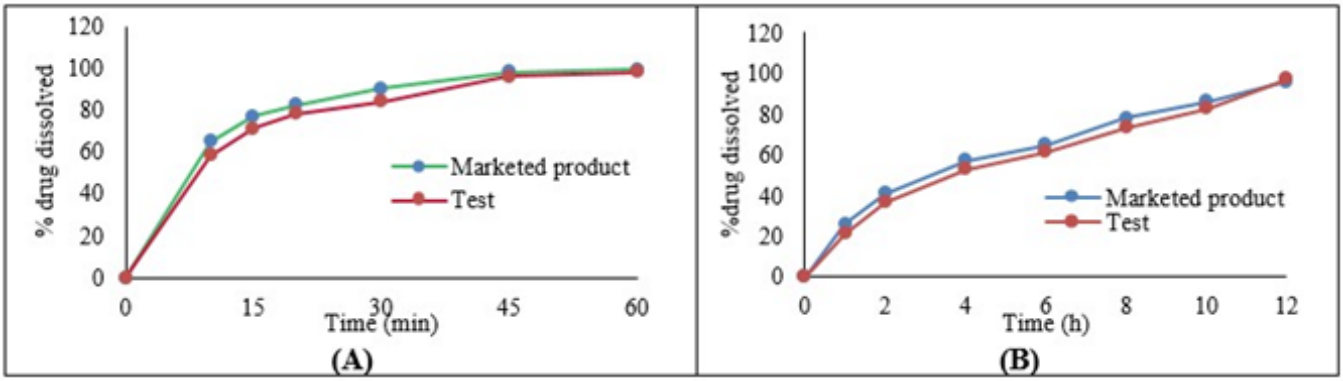

Figure 3: Comparative dissolution profile empagliflozin and metformin hydrochloride ER tablets. (A): Empagliflozin part; (B): Metformin hydrochloride part.

Table 6: *In vitro* evaluation of empagliflozin, linagliptin and metformin hydrochloride ER tablets

Component	*Empagliflozin*		*Linagliptin*		*Metformin hydrochloride*		
Assay (%)	100.5		99.8		100.8		
Dissolution	% drug dissolved (Mean ± RSD)						
Time (min)	Marketed product (Empagliflozin IR tablets)	Test (FDC)	Marketed product (Linagliptin IR tablets)	Test (FDC)	Time (h)	Marketed product (Metformin SR tablets)	Test (FDC)
0	0	0	0	0	0	0	0
10	65 ± 1.32	56 ± 2.13	61 ± 2.21	57 ± 3.10	1	26 ± 3.41	22 ± 3.25
15	77 ± 1.02	72 ± 1.65	75 ± 3.21	71 ±1.52	2	41 ± 2.56	34 ± 3.21
20	82 ± 1.23	76 ± 1.02	84 ± 1.21	79 ± 2.31	4	57 ± 3.41	51 ± 2.65
30	90 ± 0.23	86 ± 2.01	88 ± 1.51	85 ± 1.52	6	65 ± 2.36	60 ± 2.89
45	98 ± 0.52	97 ± 1.54	97 ± 0.57	95 ± 0.52	8	78 ± 1.84	73 ± 1.52
60	99 ± 0.41	102 ± 0.53	101± 0.42	99 ± 0.41	10	86 ± 1.75	85 ± 1.05
				69	12	96 ± 0.85	98 ± 0.52
f2		60	--				
				f2		66	

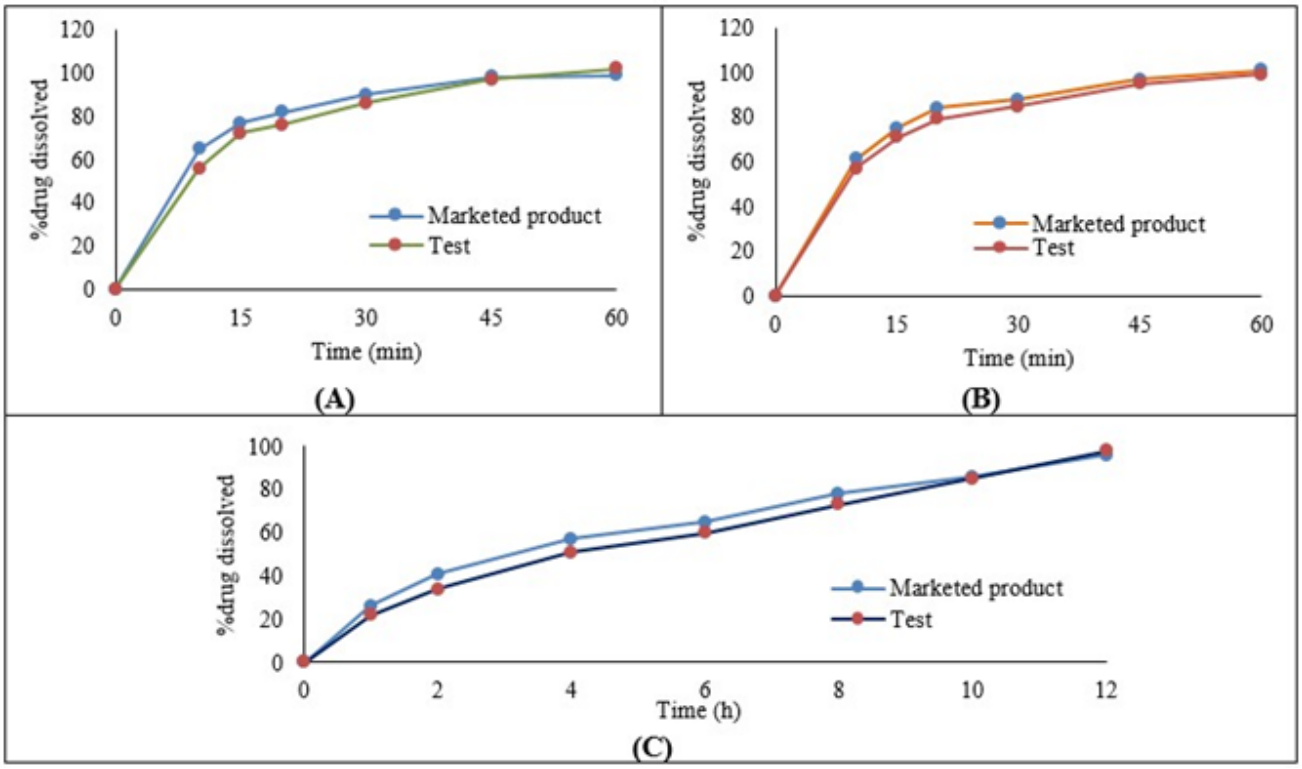

Figure 4: Comparative dissolution profile empagliflozin, linagliptin and metformin hydrochloride ER tablets. (A): Empagliflozin part; (B): Linagliptin part; (C): Metformin hydrochloride part.

Table 7: Stability results of empagliflozin and linagliptin IR tablets

Stability condition		40°C/75% RH				25°C/60% RH	
Testing Frequency		Initial	1 M	3 M	6 M	3 M	6 M
Assay (%)	Empagliflozin	99.7	100.1	99.5	100.3	99.5	99.8
	Linagliptin	100.2	100.8	100.0	100.4	99.8	100.2
Dissolution–Empagliflozin							
Time (min)		% drug dissolved (Mean ± RSD)					
30		88 ± 0.41	87 ± 1.05	89 ± 0.55	88 ± 1.02	90 ± 0.68	87 ± 0.74
Dissolution–Linagliptin							
Time (min)		% drug dissolved (Mean ± RSD)					
30		87 ± 0.89	89 ± 0.45	91 ± 0.52	88 ± 0.62	88 ± 1.02	89 ± 1.41

Table 8: Stability results of empagliflozin and metformin hydrochloride ER tablets

Condition	*40°C/75% RH*				*25°C/60% RH*	
Testing Frequency	Initial	1 M	3 M	6 M	3 M	6 M
Empagliflozin	99.4	99.1	99.6	100.1	99.7	100.2
Assay (%)						
Metformin	99.6	99.7	99.1	99.4	100.3	99.4
Dissolution–Empagliflozin						
Time (min)	% drug dissolved (Mean ± RSD)					
30	84±1.03	85±1.45	84±1.02	86±0.67	86±2.11	84±0.72
Dissolution–Metformin						
Time (h)	% drug dissolved (Mean ± RSD)					
1	21±1.06	22±1.85	22±1.02	23±1.31	23±1.34	22±1.42
4	53±1.07	52±0.64	54±0.85	53±1.94	52±1.50	52±1.61
10	83±0.96	84±1.94	84±0.52	82±0.84	84±0.96	83±1.41

Table 9: Stability results of empagliflozin, linagliptin and metformin hydrochloride ER tablets

Condition		*40°C/75% RH*				*25°C/60% RH*	
Testing Frequency		Initial	1 M	3 M	6 M	3 M	6 M
Assay (%)	Empagliflozin	100.5	99.7	99.6	100.1	100.0	99.7
	Linagliptin	99.8	100.2	100.1	99.4	99.7	99.9
	Metformin	100.8	100.4	100.8	100.2	100.0	99.8
Dissolution–Empagliflozin							
Time (min)		% drug dissolved (Mean ± RSD)					
30		86 ± 2.01	84 ± 1.52	85 ± 0.42	87 ± 0.51	84 ± 1.02	85 ± 0.56
Dissolution–Linagliptin							
Time (min)		% drug dissolved (Mean ± RSD)					
30		85 ± 1.52	86 ± 1.58	87 ± 0.78	85 ± 1.52	86 ± 1.62	84 ± 1.03
Dissolution–Metformin							
Time (h)		% drug dissolved (Mean ± RSD)					
1		22 ± 3.25	21 ± 0.52	23 ± 1.57	22 ± 1.42	24 ± 1.52	23 ± 1.55
4		51 ± 2.65	50 ± 0.42	49 ± 0.41	51 ± 1.25	50 ± 1.05	51 ± 1.23
10		85 ± 1.05	86 ± 1.55	87 ± 1.72	85 ± 0.43	86 ± 0.85	84 ± 1.53

Table 10: Fasting blood glucose levels of fixed dose combination tablets and individual drugs in diabetic rabbits

Group	*Group-A: Normal control*	*Group-B: Diabetic control*	*Group-C: Empagliflozin solution*	*Group-D: Linagliptin solution*	*Group-E: Metformin solution*	*Group-F: Empagliflozin and Linagliptin IR tablets*	*Group-G: Empagliflozin and metformin ER tablets*	*Group-H: Empagliflozin, Linagliptin and metformin ER tablets*
Time (h)	Mean fasting blood glucose level (mg/dl) ± SD, n=6							
0	92 ± 1.8	271 ± 2.1	284 ± 2.7	274 ± 1.6	276 ± 2.1	289 ± 1.9	286 ± 1.1	279 ± 2.1
1	93 ± 2.1	273 ± 1.3	262 ± 1.3	223 ± 1.7*	262 ± 2.6	231 ± 1.5	246 ± 0.8	225 ± 2.6
2	97 ± 2.6	268 ± 2.0	240 ± 2.5	198 ± 2.1*	254 ± 2.2	205 ± 1.1*	237 ± 2.1	188 ± 1.8*¥
4	95 ± 2.1	285 ± 1.5	214 ± 3.4*	213 ± 1.6*	225 ± 1.5*	184 ± 1.5*#	207 ± 1.8*	172 ± 1.7*¥
6	87 ± 1.1	281 ± 1.9	208 ± 1.6*	225 ± 1.7*	205 ± 1.6*	178 ± 0.6*#	182 ± 1.2*$	165 ± 1.2*¥
8	99 ± 1.6	275 ± 3.2	214 ± 1.1*	234 ± 2.1	190 ± 1.1*	189 ± 0.5*#	165 ± 1.5*$	145 ± 0.5*¥
10	89 ± 2.2	286 ± 1.6	221 ± 0.9*	242 ± 1.7*	187 ± 1.5*	203 ± 1.2*#	167 ± 0.8*$	131 ± 0.6*¥
12	95 ± 1.3	262 ± 2.9	239 ± 2.5*	258 ± 1.5	208 ± 0.9*	218 ± 1.4*	179 ± 0.6*$	135 ± 1.4*¥
24	87 ± 1.5	274 ± 1.5	246 ± 1.4	254 ± 0.9	224 ± 0.5*	232 ± 1.6*	195 ± 1.6*$	138 ± 1.2*¥
36	93 ± 1.3	270 ± 1.0	256 ± 2.3	261 ± 0.5	235 ± 0.4	242 ± 2.1	209 ± 1.0*$	149 ± 0.4*¥
48	96 ± 1.7	283 ± 2.2	265 ± 2.0	268 ± 1.2	248 ± 1.4	251 ± 0.5	224 ± 1.3	174 ± 2.1*¥
72	92 ± 1.5	280 ± 1.9	275 ± 2.4	270 ± 1.4	242 ± 1.6	244 ± 1.1	228 ± 1.6	184 ± 1.6*¥

*Values are statistically significant when compared to group-B; # Values are statistically significant when compared to group-C and D; $ Values are statistically significant when compared to group-C and E; ¥Values are statistically significant when compared to group-C, D, E, F and G.

profiles are more than 50 which indicates combination FDC products and individual marketed products drug release profiles are similar. The dissolution data of ER part was fitted into kinetic models and obtained results concluded that ER part drug release profiles followed the zero-order kinetics and the mechanism of drug release was non-fickian diffusion.

Stability Studies

Stability data of all 3 combination products are presented in Tables 7-9. Stability results indicate that there was no significant change in assay and dissolution profile at both accelerated and long-term stability conditions for 6 months.

Comparative Evaluation of Hypoglycaemic Activity in Rabbits

Comparative fasting blood glucose levels of fixed dose combination tablets and individual drugs in rabbits are summarized in Table 10 and graphical presentation is given in Figure 5. Comparative %reduction in fasting blood glucose levels of FDC tablets and individual drugs treated groups is presented in Figure 6. The data was subjected to student t-test and considered significant ($p < 0.05$) results. From the obtained results, it was concluded that the combination tablets have shown better hypoglycaemic activity than individual drugs. Empagliflozin, linagliptin and metformin hydrochloride ER

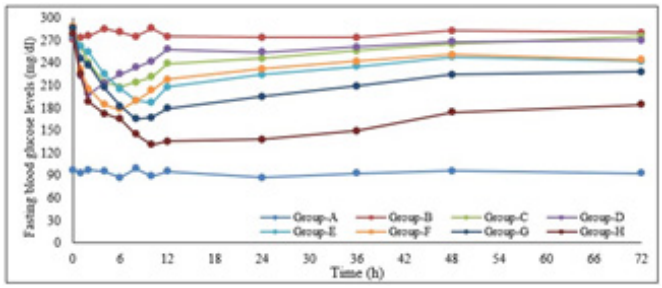

Figure 5: Comparative fasting blood glucose levels of fixed dose combination tablets and individual drugs

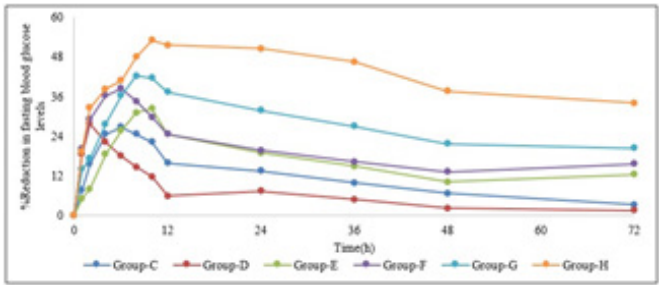

Figure 6: Comparative percent reduction in fasting blood glucose levels of fixed dose combination tablets and individual drugs treated groups

tablets have shown synergetic affect and observed improved glycaemic control than other two combination products.

CONCLUSION

Fixed dose combination tablets were fabricated and optimized formulations were evaluated for *in vitro* and *in vivo studies.* Dissolution profiles of FDC tablets were comparable with individual reference products and no significant changes were observed during stability studies at accelerated and long-term conditions. Hypoglycaemic activity of FDC tablets and individual agents were evaluated in diabetes induced rabbits. Hypoglycaemic activity of fixed dose combination tablets is effective and had shown better glycaemic control than individual monotherapies. Fixed-dose combination of empagliflozin, Linagliptin and metformin ER tablets have shown synergistic affect and resulted in improved glycaemic control than other two combination products. Fixed dose combination tablets provide a simplified treatment regimen, better glycaemic control and optimized cost-effectiveness for the management of type 2 diabetes mellitus.

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